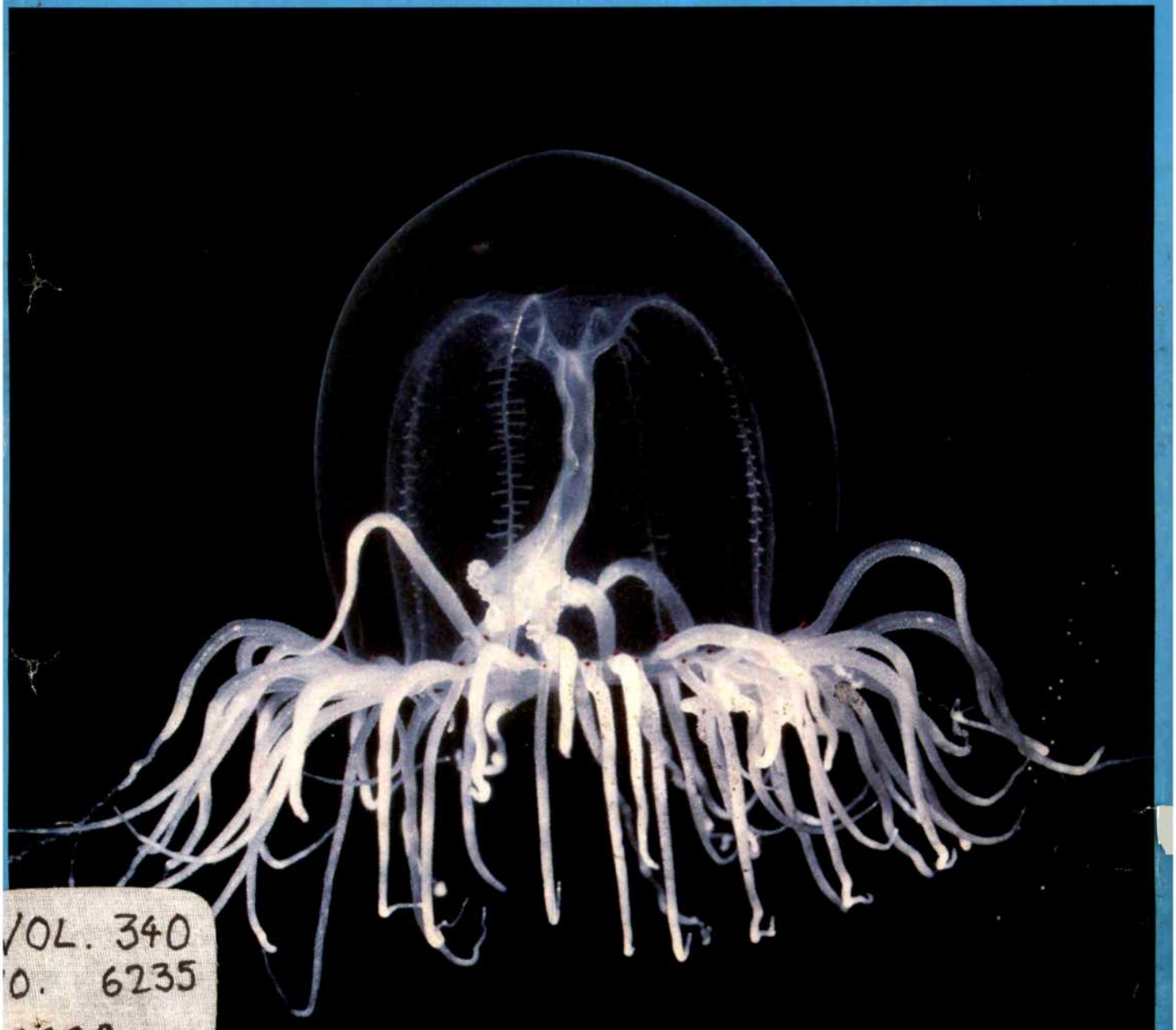
nature

INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

Volume 340 No. 6235 24 August 1989



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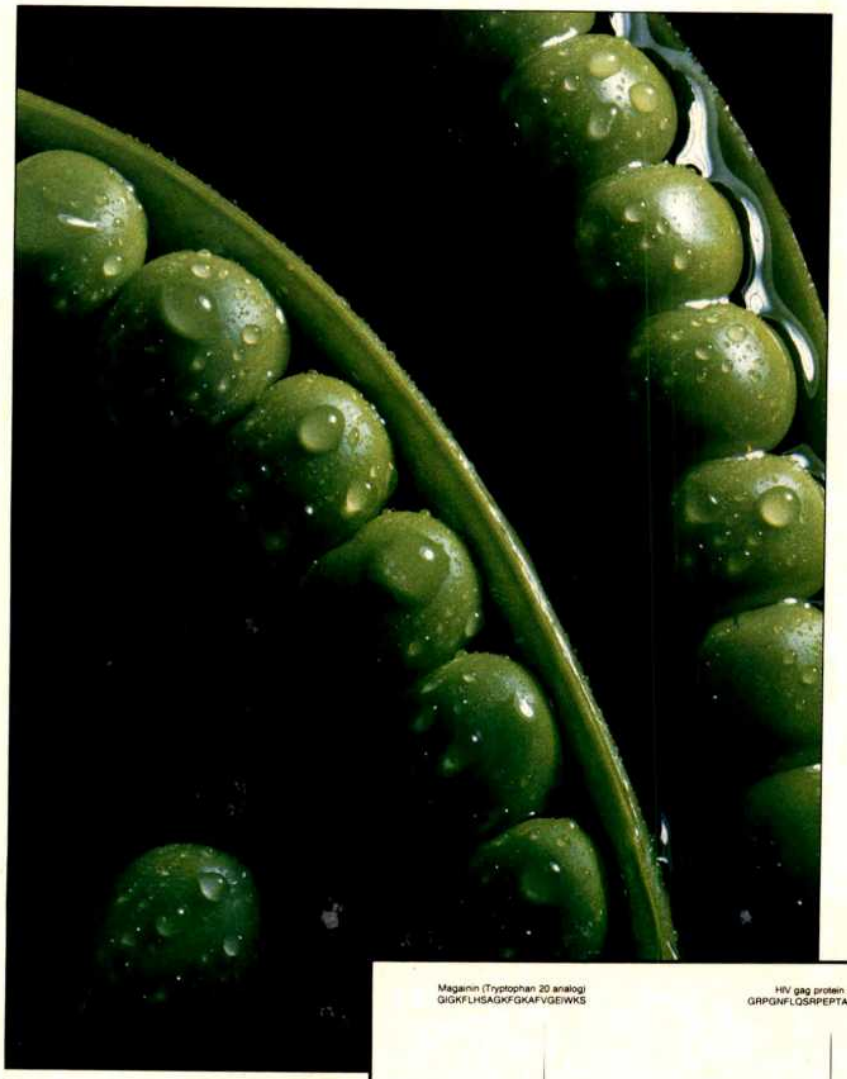

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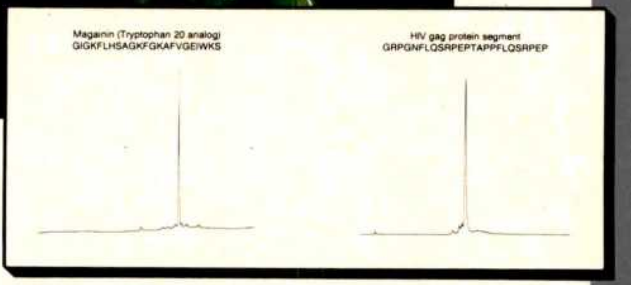

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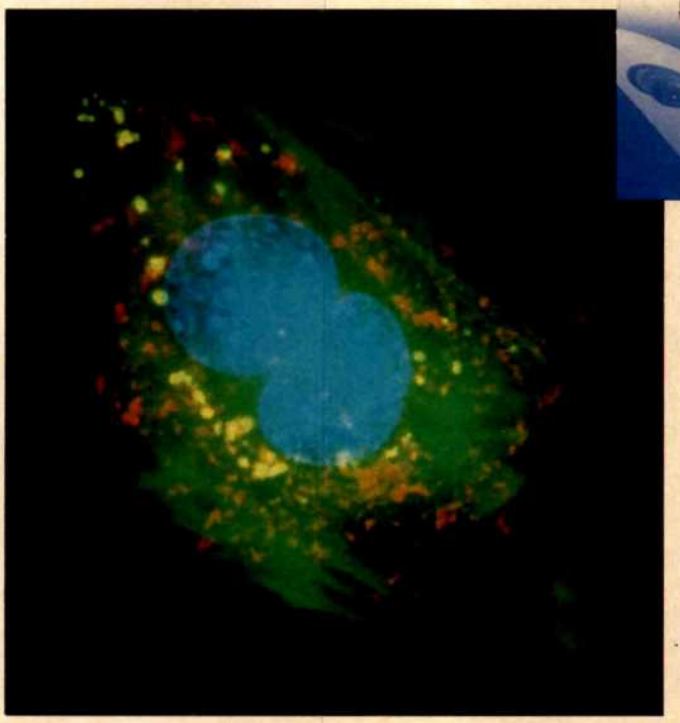

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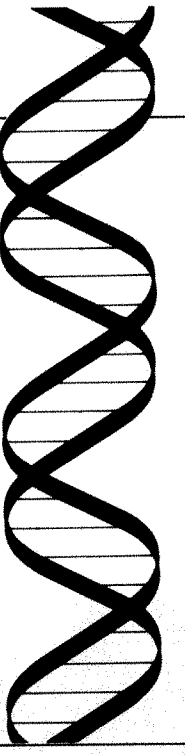

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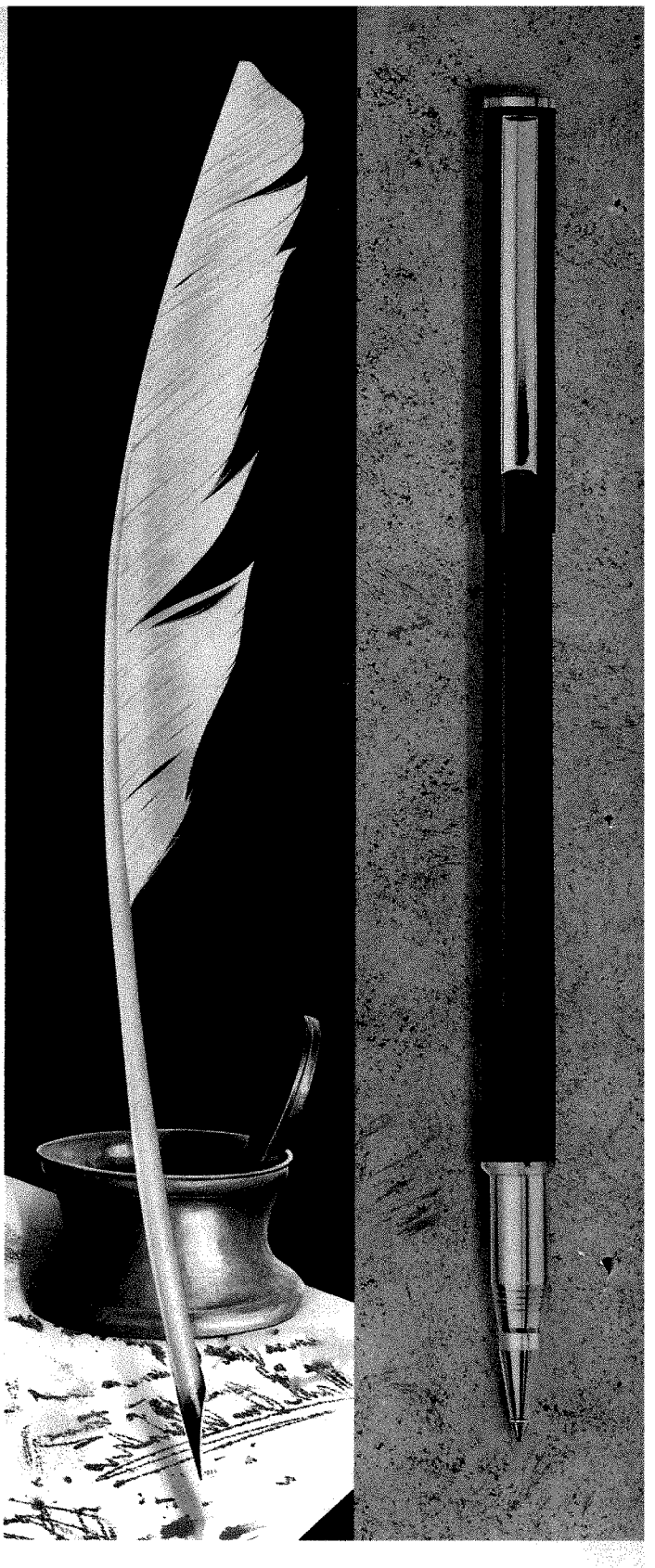

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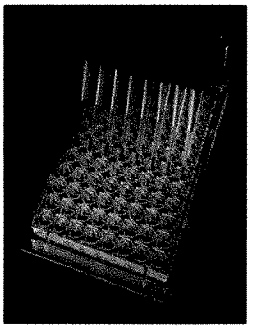

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24 August 1989

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◀ The jellyfish *Polyorchis pencillatus*, photographed by Juan Acosta. Work described on page 636 and in News and Views shows that in jellyfish motor neurons, variations in the shape of presynaptic action potentials can influence synaptic transmission.

THIS WEEK

Power from light

Solid-state photovoltaic devices achieve only limited conversion efficiency, but cells based on a semiconductor/liquid junction, like that described on page 621, have the potential to improve on the conventional devices.

cAMP followers

The DNA sequences involved in the regulation by cyclic AMP of gene expression in bacteria and eukaryotes have striking functional similarities, page 656.

In-depth analysis

Deep-sea sensors, video cameras and seafloor vehicles have been used to establish that the lowest 0.1 mm of deep-sea bottom-water is a 'sublayer' where turbulence is minimal and molecular diffusion is the dominant mechanism of solute exchange. This has implications for the study of deep-sea processes from manganese nodule formation to growth of benthic microorganisms. Page 623.

The allosteric transition

A comparison of the crystal structures of glycogen phosphorylase *b* in its relaxed and tense conformational states reveals the subtle changes that lead to allosteric regulation of the enzyme's activity. Page 609.

Biological detergent

Membrane-bound proteins can be crystallized in the presence of a detergent, which seems to bind to the protein's surface in a way analogous to the membrane itself. The determination of the structures formed by detergent in crystals of a bacterial photosynthetic reaction centre using low-resolution neutron diffraction now shows how the interaction between detergent and protein facilitates crystallization. Pages 659 and 601.

On the rise

Marine seismography data collected along the East Pacific Rise are interpreted to show that 'sills' of molten material occasionally accumulate at the base of young ocean crust. Escape of this melt to the seafloor could be responsible for the formation of off-axis seamounts. Page 628.

Where's the catch?

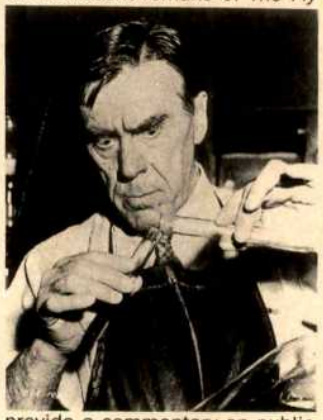
The identification of the factors controlling the variability of fish populations would be of great value to those responsible for fisheries policy. The North Sea herring, which suffered a collapse in population in the 1970s, provides a model. Page 632.

Doing it with mirrors

If attempts to moderate emissions of greenhouse gases fail, decreasing solar radiation may be the answer. Scientific Correspondence, page 603.

An image problem

Horror movies from the 1930s to the recent remake of *The Fly*



provide a commentary on public anxieties about science and technology. Andrew Tudor takes his seat in the stalls, page 589.

Guide to Authors

Facing page 604. Also Vol. 340 page 408.

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P 36,674

CORRESPONDENCE

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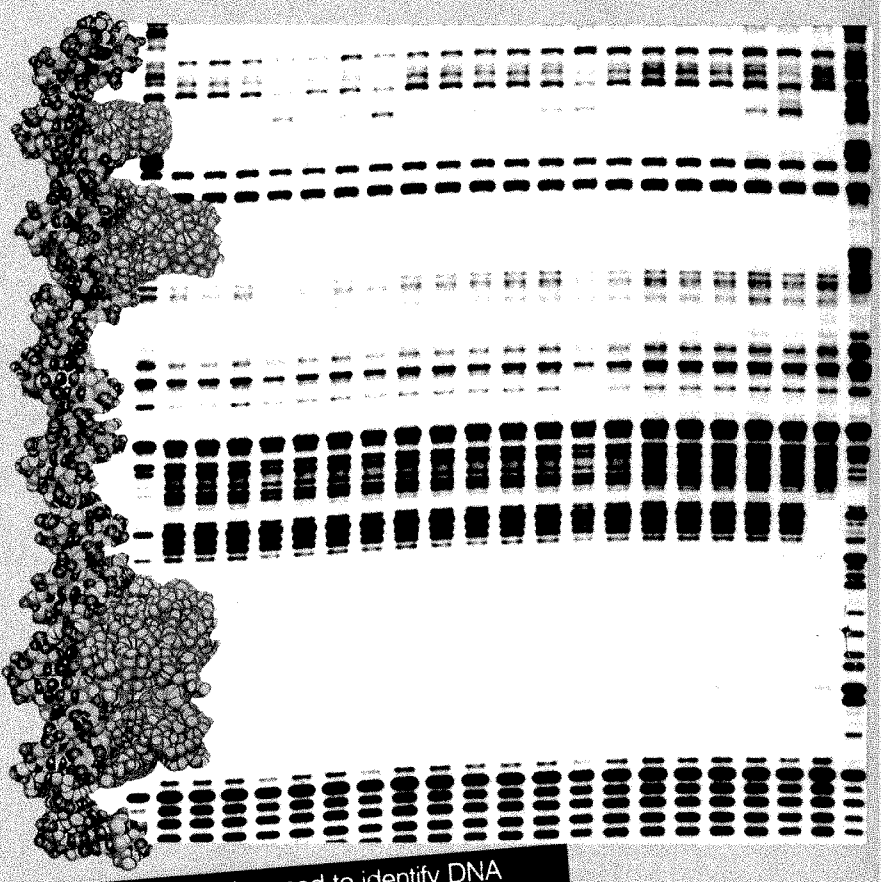

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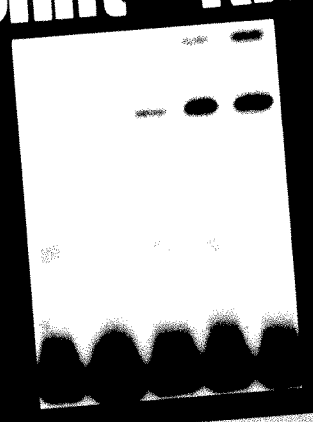

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Competition experiments can also be used for estimating the abundance of experimental protein in various extracts.

(1) Hendrickson, W. (1985). Biotechniques 3, 198-207

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Nuclear activity in galaxies ■ Accelerating pulsars ■ Sea-floor venting systems ■ Spiders webs as early as the Cretaceous ■ Interspecies competition ■ Magnetic separation of DNA

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Vol. 340 No. 6235 24 August 1989

NATURE VOL 340 nature 24 AUGUST 1989

How can polluters be made to pay?

The doctrine that polluters and other environmental desperadoes should be required to pay for the damage they do is beguiling, but first requires general agreement on broad philosophical principles unlikely soon to be reached.

Dr David Pearce, professor of economics at University College, London, scored a big success last week when the new British Secretary of State for the Environment, Mr Christopher Patten, cautiously approved a report which, among other things, argues that the rational protection of the environment requires a system by which environmental assets and natural resources are valued financially, so that their use (or the damage done to them) can be taxed on an equitable basis. The proposition appears to be equitable both between people now alive and those likely to be alive at future epochs. But it is unworkable except in the most restricted fields.

That polluters should pay is a sound principle, nominally the basis of British environmental policy for decades, but more often honoured in the breach than the observance. Although Patten's predecessors have paid lip-service to it, they have usually found that there is no sound basis on which charges can be levied. In principle, the managers of a water-course might argue that those discharging effluents should pay charges proportional to the biological oxygen demand (BOD) of their materials. But what is to be done about inorganic pollutants, for example? And should latecomers to a river-bank pay more than early arrivals because of the probable non-linear effects in the absorptive capacity of the water-course? In Britain, in practice, the application of even this simple principle is too often thwarted by the fact that the managers of watercourses (the statutory water boards) are also the chief sources of BOD (as sewage).

The problems are more serious when comparing different kinds of pollution, for example the discharges to the atmosphere from nuclear power stations and those burning fossil fuel. Nuclear power stations unavoidably generate quantities of radioactive isotopes of the rare gases, mostly argon, which contribute to radioactivity in the atmosphere and which as a consequence may increase the incidence of lung cancer, but to a degree that is hardly large enough to be called marginal compared with natural causes of radiation (leaving cigarette-smoking aside). But fossil-fuel power generation is much more hazardous: apart from being one cause of acid rain, it is also a substantial cause of ill-health, perhaps even premature death, from bronchial diseases. But what politician, these days, is likely to take up the cause of taxing nuclear generating plants less heavily than more old-fashioned plants, however compelling the logic?

The balance between one generation and its successors is even harder to strike sensibly. Take, for example, the present use of petroleum, the reserves of which are finite (in the sense that the quantities that could be produced at present prices must be limited). So it is possible to argue that the present generation should tax petroleum so as to discourage its use, leaving some part of the present stock for the next generation. But that would turn out to be a self-defeating self-denial if, in the next century or so, some other substantial source of energy makes its appearance. And, quite apart from thermonuclear fusion, are there not solar power, wave energy and windmill energy at the front of many people's minds?

The essential weakness of the Pearce proposition is that, while it may make sense in restricted fields, it quickly becomes a nonsense when there is no objective basis for evaluating the damage done by pollution or other environmental insults. There can be no substantial basis, for example, for putting a price on the continued survival of whales or tigers, but such a price would probably be negative (whales eat krill, which might otherwise support commercial fisheries, while tigers kill people among other creatures). And there is a further weakness underlying this beguiling but illfounded argument: since the ultimate cause of environmental damage is people, should births be taxed? And on what basis? □

Poland's way ahead

The new regime in Poland has to walk a delicate tightrope. Here is how well-wishers can help.

The irony that the appointment of Poland's first Solidarity prime minister (but Mr Tadeusz Mazowiecki has not yet formed a government) should almost coincide with the fiftieth anniversary of the treaty between the Soviet Union and the Third Reich might almost have been deliberate. That treaty is now acknowledged to have included a hitherto secret protocol in which the two signatories agreed on the partition of Central Europe, and of Poland in particular. A shrunken Poland has been part of the Eastern bloc, and a member of the Warsaw Pact, ever since. Well-wishers should keep in mind that it will remain so for a very long time, perhaps indefinitely.

That, of course, is why Polish politicians have been

treading so delicately in the past few dramatic weeks. Solidarity, which began life as a trade union representing shipyard workers in Gdansk, and which has been illegal for much of its time, has shown all the signs of not wishing to form Poland's next government. It has been forced into accepting what many regard as a poisoned chalice (but it is not) by the failure of the Polish Communist Party to do so, which is in turn a consequence of the unwillingness of the Polish parliament (called the Sejm) to accept the arrangements proposed. That circumstance will itself surprise many in the West with an over-simple view of how the governments of Eastern Europe are organized. Although the Sejm may have been newly legitimized by last June's semi-free elections, it is at least a parliament that, on this occasion, has shown independence.

Luckily, everybody in Poland seems fully aware of the difficulties the developments of the past week have created for the other members of the Warsaw Pact, the Soviet Union included. In the real world, Mazowiecki cannot risk signs of wishing to leave the Eastern bloc without bringing down not only himself but also Mr Mikhail Gorbachev. The practical problem that he faces is whether he can win enough freedom to deal with Poland's pressing economic problems within a framework in which his motives will be deeply suspect. A firm assurance of indefinite devotion to Comecon, Eastern Europe's less democratic version of the Europen Community, is a necessary precondition of survival.

What, in the circunmstances, can people elsewhere do? The conventional view is that there is little room for manoeuvre. But that is what governments say. Offers of large amounts of credit to help with Poland's economic difficulties could easily become, in a few months or years, unpaid loans to a Polish government of quite a different complexion. Even so, there is a case for much more generosity than has been offered so far, if only to sustain hope during the harsh winter that lies ahead. But this is also an unprecedented time, when an apparently sovereign state appears openly to acknowledge that it is at a loss to know how to get out of the mess in which it finds itself. These may be circumstances in which the intellectual community in the West is more able to respond imaginatively than Western governments. A decade ago, the Brandt Commission made some constructive suggestions about the place of developing countries in the modern world, and had an important influence on public policy. Has the time come for the equivalent of a Brandt Commission for Eastern Europe? □

Funny money

British ambitions to find a better convertible currency than the European Commission's await fulfilment.

WESTERN Europe needs to invent some kind of currency that can be used in all 12 member countries of the European Community by the end of 1992, but has not yet agreed how that should be done.

More precisely, the European Commission last year proposed that the European Currency Unit (ECU) should become a common currency, and that there should a Central Bank to manage its affairs, but the British government objected at June's summit meeting in Lisbon, since when West Germany has been having second thoughts. But the British government's promise to devise an alternative scheme has not yet lived up to realistic expectations, at least to judge from the British Treasury's whispered information at the weekend.

That there are serious objections to the Commission's proposals cannot be denied. Much would depend on the degree of independence enjoyed by the proposed Central Bank. If, for example, it had a large measure of autonomy, as does the Federal Reserve Bank in the United States or the Bundesbank in West Germany, there would be endless complaints from member governments that the bank was following over-conservative (or over-open) policies. If, on the other hand, the bank were politically controlled, there would be endless squabbling among the members about the correct policy to be followed. European hardliners say, with some justice, that such difficulties are inseparable from the goal of economic unity.

The British government's alternative has the merit of being imaginative, even intriguing. The principle is that existing national currencies should be allowed to compete with each other in the market places in which goods of various kinds are traded for banknotes of different denominations, but that all European currencies should simultaneously be legal tender everywhere. The argument is that national prestige would persuade governments to manage their currencies in such a way that they were considered sound, and that the soundest currency would eventually prevail.

But that is an over-sanguine view. While sellers of goods would have an interest in being paid in the currency most likely to keep its value from one day to the next, purchasers would have exactly the opposite interest. A kind of Gresham's Law would apply.

Quite apart from the horrendous difficulties arising under the British government's proposals when people from different parts of Europe sought to buy cups of coffee and other trivial goods in their national currencies, there are bound to be serious objections to any scheme that tempts either party to a commercial bargain to argue about the currency as well as the price. And would banks as well as traders be required to convert one currency into another without charge, which would be equitable in the circumstances? But there are merits of flexibility in the British proposals. The best compromise would be simultaneously to make the ECU and individual national currencies legal tender everywhere, thus allowing those who buy and sell things in Western Europe to deliver a vote of confidence in their own governments' conduct of economic affairs whenever they go into a shop. In the last resort, that would also be a constraint on the conduct of the otherwise inevitable European Central Bank. □

AIDS closer to becoming a treatable disease

- Antibody-CD4 chimaera in clinical trials
- AZT effective in most cases of HIV infection

Washington

AUGUST has so far been a good month for news of AIDS therapies. Three weeks ago, AZT was shown to delay the progression of AIDS in those with AIDS-related complex, and last week it was shown to postpone the development of symptoms in those whose T-cell counts had dropped below 500. This week, clinical trials are set to begin of another emerging treatment that could be used with AZT to lengthen the lives of those infected with HIV (human immunodeficiency virus). Together with AZT, the hybrid antibody-CD4 molecule termed 'immunoadhesin' offers hope that HIV infection may in future be treated as a chronic disease that can be managed for years before it ultimately become fatal.

Immunoadhesin is a genetically engineered molecule composed of part of the CD4 receptor — the entry point through which HIV infects cells — and the back end of the IgG1 antibody, responsible for activating killer cells in the immune system and initiating a virus-attacking system within the blood named complement. Genentech, the company developing immunoadhesin, has shown that it blocks the infection of CD4-bearing cells in culture, and stays in the blood of rabbits for more than four days (see *Nature* **337**, 525; 1989).

Soluble forms of the CD4 receptor alone — being tested by Genentech and Biogen — stay in the bloodstream for only roughly an hour when injected directly into the veins of humans. Partly because it is larger, immunoadhesin is expected to last 20–50 times longer, requiring fewer injections for patients. Unlike plain soluble CD4, immunoadhesin may also kill infected cells and traverse the placental barrier, allowing it to be used to protect the fetuses of HIV-infected pregnant women.

The clinical trial of immunoadhesin will be conducted by Genentech's collaborators at the US National Cancer Institute (NCI), the New England Deaconess Hospital in Boston, Massachusetts, San Francisco General Hospital, the University of Washington and Stanford University. The drug will be tested for safety in a total of 30–40 patients given a range of dosages intravenously. The first patient will be treated this week by Samuel Broder's group at NCI — just six months after the initial publication of immunoadhesin, a speed Broder says is "astonishing". If the results are encouraging, efficacy studies could begin within a year.

Genentech is now planning controlled efficacy trials for its first-generation soluble CD4. Since the federal approval of AZT two years ago, all drugs being tested in people with AIDS have been compared to AZT, limiting placebo-controlled trials to those with a less advanced stage of HIV disease. But the announcements that AZT is effective in people with AIDS-related complex and in some asymptomatic HIV-infected individuals will mean that AZT will now be the standard of comparison for nearly all trials of drugs to treat HIV disease.

The manufacturer of AZT, Burroughs-Wellcome, is expected to use data from the new AZT studies to ask the US Food and Drug Administration to broaden the conditions the company can specify in the drug's labelling. Anticipating that AZT will now be used to treat a much wider spectrum of those with HIV disease, stockholders bid up Wellcome plc's stock price by 32 per cent by the end of last week.

But the drug still costs $8,000–$10,000 for a year's treatment at a full dose. Jude Payne of the Health Insurance Association of America says "almost all" US health insurers will begin paying for AZT for those with AIDS-related complex and HIV-infected people with T-cell counts of less than 500, based on the new studies. Senator Ted Kennedy (Democrat, Massachusetts) plans to add an amendment to next year's appropriations bill for the US Department of Health and Human Services which would provide $30 million for three years to pay for AZT for people with AIDS who have no insurance. When Congress returns from its summer recess, there will be pressure for Kennedy's amendment to be extended to cover all those with HIV infection.

Carol Ezzell

HIPPARCOS

ESA tries to salvage Hipparcos

Munich

AFTER nearly two weeks of unsuccessful attempts to fire the apogee motor on the European astronomy satellite Hipparcos, project leaders have reluctantly begun to design a salvage operation for the mission. The 1,140-kg satellite is currently in an elliptical orbit between 200 km and 35,000 km from the Earth. Project scientist M.A.C. Perryman says the chances are "very small" that Hipparcos can be brought into a geostationary orbit by the motor, as was originally intended (see *Nature* **340**, 491; 1989).

One or two more attempts to fire the motor will be made on 24 or 25 August. If they fail, small thrusters on the satellite will be fired early next week to bring the satellite into a higher orbit at a minimum distance of about 600 km from Earth. The thrusters were originally meant to be used to correct for nutation (wobbling) once the satellite was in geostationary orbit.

Hipparcos was designed to measure the precise positions, parallaxes and proper motions of stars. It was expected over two and a half years to measure the positions of 120,000 stars with a precision of 0.002 arcseconds. A second mission on board, called Tycho, was meant to measure the positions of 400,000 stars with an accuracy of 0.03 arcseconds by 1995.

In the current orbit, Hipparcos could last from six months to two years, depending on how fast the solar cells that power the satellite degrade. In six months, the satellite could achieve only one-third the accuracy initially projected, although this would still improve on ground-based results by a factor of 15.

ESA has not decided to ask its 13 member states for the money to build and launch a second Hipparcos satellite identical to the first. But a preliminary decision is expected "in one month", said Roger Bonnet, director of the ESA scientific programmes, after the extent of the degradation of the solar cells has been determined. The ESA council would have to approach the scientific programme committee to request more money. A replacement satellite would cost an estimated £114 million ($182 million).

It would be difficult to reapportion the money out of the existing ESA science programme budget, which would require a two-thirds majority vote in the committee. But it would be even more difficult to gain a new appropriation for a second Hipparcos, which would require unanimous support. The length of time in orbit is crucial. In six months, only 8 or 10 observations of each star would be possible instead of the 80 originally planned. If Hipparcos were to survive longer, say for a year or a year and a half, proper motions of stars and parallaxes could begin to be measured. But Perryman said that was not a "realistic" expectation.

In its elliptical orbit, the satellite passes every ten hours through the Van Allen belts, where it is subject to irradiation by protons and electrons in the solar wind 5,000 km above the equator. This radiation degrades the solar panels which power the satellite.

Steven Dickman

DNA FINGERPRINTING

Judge backs technique

Washington

IN the New York double-murder trial that has become a test case for the forensic use of DNA 'genetic fingerprinting', Justice Gerald Sheindlin last week ruled that the identification techniques are generally reliable and admissible as evidence. Genetic fingerprinting thus emerges from what Sheindlin described as its "most comprehensive and extensive legal examination . . . in the United States" with backing for its use both to demonstrate exclusions (that two samples are not the same) and inclusions (the more difficult test that two samples are identical).

The decision comes with the recommendations that, in future trials, "a pre-trial hearing should be conducted to determine if the testing laboratory substantially performed the scientifically accepted tests and techniques, yielding sufficiently reliable tests to be admissible". In the particular case under examination, Sheindlin ruled that the testing laboratory had, on one inclusion test, "failed in several major respects to use the generally accepted scientific techniques and experiments for obtaining reliable results".

Peter Neufeld, one of the defence lawyers in the case, believes that despite the new recommendations, the judge "did not go far enough" in his criticisms. When the judge decided the evidence was in a general sense reliable, Neufeld says, he was "responding to the hopefulness of our expert witnesses rather than their assessment that these standards and procedures were currently in place". But Neufeld adds that, as the case represents "the very first time that any judge has excluded DNA evidence of a match, on the grounds that the tests were not done in a sufficiently reliable fashion, it has tremendous impact on testing laboratories nationwide".

The DNA identification evidence is of vital significance in the trial of Joseph Castro, a 38-year-old resident of the Bronx, accused of stabbing to death a 20-year-old pregnant woman and her two-year-old daughter. A wrist-watch worn by Castro at the time of his arrest appeared to carry a bloodstain. Castro claimed that the blood was his own. DNA identification tests were performed by the forensic and paternity testing company, Lifecodes Corporation of Valhalla, New York, and were claimed to show that the blood came from the murdered woman rather than Castro.

A pre-trial hearing examined whether the scientific procedures involved met the 'Fyre' test of being "generally accepted as reliable" by the scientific community. The hearing quickly turned into an intense examination of forensic DNA tests in general and of the methods used by Lifecodes Corporation in this particular case.

Approximately 5,000 pages of evidence were amassed over the 12 weeks and a series of distinguished scientists testified for the prosecution and for the defence. The hearing took an unusual twist when defence and prosecution witnesses got together and agreed that some of the evidence was not scientifically reliable.

Although there was speculation that the judge might rule DNA testing inadmissible, the final decision accepts its general validity. In the particular case under consideration, the ruling accepted that Lifecodes Corporation showed that the blood on the watch did not come from the defendant. But the judge ruled inadmissible the company's evidence that the blood matched that of the victim.

Despite the implied criticism of their procedures, Lifecode's senior vice-president John Winkler said he was "delighted that the judge recognized that using DNA for forensic testing is valid". Winkler also accepted the judge's view that "a preliminary, critical examination of the actual testing procedures performed in a particular case" should be conducted before the start of a trial employing DNA identification tests. The judge notes that the test results are bound to have a "powerful impact" on a jury and makes twelve recommendations of what should be presented at a pre-trial hearing.

Winkler says that Lifecodes "agrees with all of the judge's recommendations". Among them are details of the method used to declare a match or nonmatch between samples, the methods used to calculate the allele frequency in the relevant population and tests performed to determine the origin of contaminants. Evidence on all these points was strongly debated during the Castro hearing (see *Nature* **339**, 501; 15 June 1989).

Comprehensive guidelines for the forensic use of DNA fingerprinting are still lacking, and the National Academy of Sciences is still hoping to find funds for a detailed study. Meanwhile the Federal Bureau of Investigation (FBI), whose laboratories see themselves as leaders in the forensic DNA analysis field, has brought together molecular biologists, testing-laboratory representatives and its own experts to exchange viewpoints.

Neufeld says, however, says that state and federal legislation is necessary, Included should be accreditation of laboratories, certification and licensing of personnel, quality control standards, and proficiency studies.

"It's absurd", says Neufeld "to think that you do not have all that in a testing procedure which determines who lives and who dies, or at a minimum who goes away to prison for 30 years".

Alun Anderson

CHINA

Japanese scientists stay clear of China for time being

Kyoto & Tokyo

JAPAN'S Ministry of Education, Science and Culture (MESC) has been quietly cutting off the flow of Japanese scientists to China in the wake of the Tiananmen Square killings. Although the ministry has made no official announcement, information on travel grant applications released earlier this month show that visits to China are no longer winning support.

Immediately after the imposition of martial law in China, Japanese government officials and academics involved in academic exchange with China said only that they were "watching the situation" and had no immediate plans to suspend their exchange programmes.

But, it has now emerged that on 6 June, MESC sent a directive to all national universities and research institutes advising against visits to China. The directive is not legally binding but has had the effect of bringing visits by Japanese academics to China to a virtual halt.

A group of mineralogists at Kyoto University led by Professor Shohei Banno had been expecting to receive a grant for a long-planned collaborative project with the the Chinese Academy of Sciences to look for unusual minerals in eastern China. But the grant was not among those announced this month. Private funds are available for the trip but because permission for leave of absence must be obtained from the university, the trip cannot go ahead.

Figures provided last week by the Japan Society for the Promotion of Science (JSPS), a semi-governmental organization funded by MESC, also show that the flow of scientists to China has fallen to a trickle. Normally about 30 to 50 Japanese scientists visit China each year under JSPS programmes. But since 4 June only one scientist has been despatched and many have postponed their trips, according to a JSPS spokesman.

The ministry is particularly anxious to avoid having Japanese scientists in Beijing, where martial law is still in force, according to Rinjin Ogasawara, a MESC spokesman on China. Grants that have already been awarded for trips to Beijing can be used for journeys elsewhere, including other countries, or they can be returned to the ministry, as some have been, Ogasawara says.

In the future, however, it seems likely that the flow of Japanese scientists to China (excluding Beijing) will resume. The foreign ministry announced last week that it is withdrawing its advice against Japanese nationals travelling to China, except Beijing, and economic assistance will also be resumed.

Simon Wallis & David Swinbanks

REMOTE SENSING

French back Spot satellite

Paris

FRANCE has decided to back its commercial remote-sensing programme until the end of the century by giving the go-ahead to build a fourth Spot satellite. The decision, announced after a cabinet meeting last week, should mean an extra FF200 million ($31 million) in next year's budget for the national space research centre (CNES).

At a time when the US Landsat remote-sensing satellites have been given only a temporary reprieve pending a review by the new Bush administration (see *Nature* **338**, 365; 1989), the French government is more than ever committed to Spot.

New political interest in environmental issues, together with France's long-standing enthusiasm for space technology, makes the Spot programme an ideal vehicle for national prestige. But Spot-1, launched in 1986, has already overrun its intended life by one year and CNES has decided to take up its option to launch Spot-2 in November aboard an Ariane 40 rocket. CNES had originally hoped to delay the launch so long as Spot-1 continued to function, in order to save money.

Spot-4 embodies some significant new features compared with its predecessors, Spot-1, 2 and 3, which will make it more useful for monitoring changes in vegetation throughout the world. Spot-1's two high-resolution visible (HRV) charge-coupled sensors will be updated and their bandwidth extended to the medium infrared. The new sensors will be made by the French company Thomson instead of the US Fairchild which supplied Spot-1, 2 and 3. The switch to a French company, say the satellite's makers, Matra-Espace, should allow better control of component quality. Interestingly, Matra last week acquired three of Fairchild's communications and space divisions. Spot-4's two data tape-recorders will also be made in France, by Enertec, instead of by the US company Odetics. One of the two Odetics recorders aboard Spot-1 has failed, leaving the satellite unable to send pictures of some areas of the globe.

The change to French manufacturers could also be because a military satellite, Helios, due to be launched in 1993, shares its chassis and many components with Spot-4. The technology will also be useful in the development of the European polar platform contribution to the Freedom space platform. These factors may have helped secure government support.

Despite the 'green' aspect of the decision to back Spot-4, it will be less useful to monitor environmental change than its original design intended. A multispectrum 'vegetation' instrument with a scanning width of 2,220 km and a resolution of 60 km, which would have made possible global change monitoring, has been dropped. Additional data-processing facilities, both within the satellite and on the ground, would have increased costs by as much as FF1,000 million.

Construction costs for Spot-4 are down to about FF3,000 million, compared with the FF5,000 million for Spot-1, and its life has been extended from two to four years. Nevertheless, Paul Quilès, minister for posts, telecommunications and space, has said that sales of Spot pictures should balance operating costs (FF210 million in 1988) by 1993.

Spot-4 is scheduled for a 1995 launch, but should be ready to take over if Spot-3, due for launch in 1992, fails. **Peter Coles**

SEISMOLOGY

US–Soviet earthquake collaboration

San Francisco

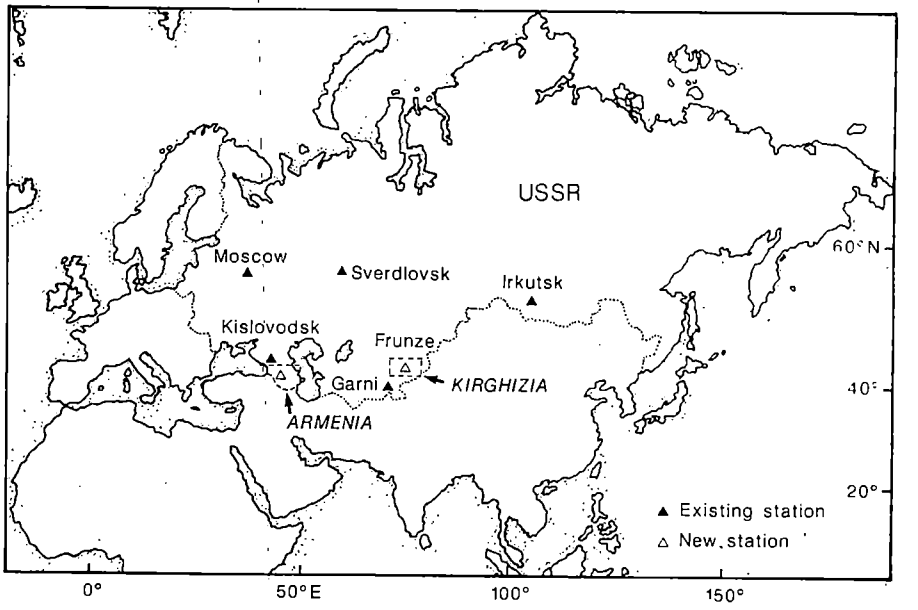
A COMPREHENSIVE scientific agreement between the United States and Soviet Union will make possible two new monitoring stations inside the Soviet Union to help fill in what has long been a 'seismic hole' in a worldwide earthquake-detection network.

The US–Eurasian Seismic Studies Program also allows the upgrading of five existing Soviet seismic listening stations. Altogether, the new efforts are expected to improve earthquake prediction and provide a much more detailed picture of how seismic waves propagate through the Earth in the region (see map). The data will also provide useful background in attempts to monitor underground nuclear tests.

The new agreement marks a joint effort between the US Geological Survey, the Soviet Academy of Sciences and the Incorporated Research Institutions for Seismology (IRIS), a consortium of more than 60 US universities. The five-year programme, supported by the Defense Advanced Research Project Agency (DARPA), includes a first-year budget of $5.7 million. Most of that money will go to the Scripps Institution of Oceanography, part of the University of California at San Diego, which will coordinate and manage all IRIS operations in the Soviet Union.

The five existing sites, scattered in a crude circle, comprise part of a 23-station global network called the International Deployment of Accelerometers that is managed by Scripps for IRIS. The new stations will fill in holes in that network by providing the first comprehensive look at two active earthquake zones. One will be installed near Frunze in the south central Soviet Union, where tectonic plates meet to form the boundary of the Indian and Asian subcontinents. The other will be in Garni, just west of the Caspian Sea near the site of the devastating earthquakes that rocked Soviet Armenia last December.

Neither station will be within 1,000 kilometres of a Soviet nuclear test ground, and will therefore have no direct bearing on the monitoring of nuclear tests, according to Scripps geophysicist Bernard Minster. But he stresses that the improved model of the Earth, and of how seismic waves travel through it, will indirectly bear on any verification process. "By learning more about the Earth we all get information that will be usable by people who worry about nuclear monitoring", Minster says.



As part of the agreement, Soviet researchers will receive data from five already existing seismic stations in the United States and two new sites to be established in New Mexico and Virginia.

Robert Buderi

Germans debate maglev train

Munich

THE future of West Germany's plans to build a magnetically levitated train called 'Transrapid' is looking increasingly uncertain. Transport Minister Friedrich Zimmermann said recently that his ministry would not spend "a single deutsche mark" on Transrapid. Zimmermann fears that the system would compete with the state-run conventional railway system, from which the government recently assumed DM12,600 million of debt.

The Research Ministry has already invested DM1,200 million (about $615 million) in the project, but it has now withdrawn its support. A further DM3,000 million in long-term loans is needed to finance the next phase.

Initial plans were for a route connecting Hamburg and Hanover, reducing the train travel time for the 155-kilometre distance from 80 minutes to just 30–40 minutes. But a second stretch in the Ruhr area linking Essen and Bonn is now being considered. The West German cabinet is expected to debate the matter in October.

The consortium to build Transrapid is led by Thyssen Henschel and includes Messerschmitt-Bölkow-Blohm and several of the largest banks in West Germany. The consortium has itself invested about DM100 million in the project so far.

Thyssen admits that Transrapid can be profitable in West Germany only if two distant cities are connected. It has recently raised the stakes by offering to build a 1,025-km track between Hamburg in the north and Munich in the south, looping past the Ruhr and Rhine valleys on the way. Such a track could cost between DM30,000 million and DM45,000 million. It would take at least 15 years to complete, not counting the time spent fighting court battles against environmentalist opponents of the project.

Although in the 1960s maglev trains were thought of as the best way to link major European cities, the technology has been turned down by the French, the British and others in favour of improving conventional railways. Even West Germany has invested heavily in improving its high-speed intercity rail network with trains that can travel up to 250 kilometres an hour.

Thyssen conducted a publicity campaign this summer to try to win support for Transrapid. Spokesman Lutz Dreesbach said that Transrapid is meant to complement the conventional rail network, not to compete. The plan to link Hamburg and Munich would also link seven major airports, providing significant relief for the overcrowded air lanes in West Germany, he said. Dreesbach argues that Japanese plans to build a maglev system should motivate West Germany to back its own superior technology.

The West Germans claim to be three or four years ahead of Japan, which they see as the main competitor in the potentially lucrative US market. Transrapid, which uses conventional magnets, last year set a speed record for a levitating train (with passengers) of 412 kilometres an hour.

Steven Dickman

Go-ahead for

Tokyo

JAPAN took a step towards developing a commercial railway line for a high-speed magnetically levitated (Maglev) train two weeks ago with a decision to locate a new 40–45 kilometre test track for the Maglev in Yamanashi Prefecture, about 100 kilometres west of Tokyo. The test track may later be extended to link Tokyo and Osaka for commercial operations. But before that many technological, economic and political hurdles have to be overcome.

The Yamanashi site was chosen by a Transport Ministry committee over two other possible sites, one in the northern island of Hokkaido, the other in the southern island of Kyushu where there is already a 7-km test track for the Maglev. The ministry will apply for ¥6,000 million ($45 million) in fiscal year 1990 towards the cost of developing the track when budget requests are submitted at the end of this month. Total cost of the test track, trains and related facilities will be about ¥350,000 million and this is expected to be shared by the national government, the local Yamanashi government and one of the Japan Railway (JR) companies, JR Tokai, which was formed after the privatization of the Japan National Railways (JNR) in 1987.

The test line will be used for manned high-speed runs of up to 500 kilometres an hour and to test the Maglev on inclines and curves and in tunnels. So far the Maglev has been tested only on the straight 7-km track in Kyushu. But before it goes into commercial operation, the Railway Technical Research Institute which developed the train will have to find a way of shielding passengers from the strong magnetic field of the trains superconducting magnets. Watches, computers and heart-pacemakers are liable to stop when inside the institute's present test train (MU 002).

Apart from these technological hurdles, there are strong forces resisting plans of Maglev proponents to extend the line to Osaka and Tokyo. An Osaka–Tokyo Maglev could whisk passengers between the two cities in an hour instead of three hours by conventional bullet train. But this would threaten the commercial base of the existing bullet train lines (*Shinkansen*).

When JNR was privatized, the company had an accumulated debt of more than ¥25 million million ($185,000 million), much of it resulting from the building of *Shinkansen*. The debt, which was passed to a semi-governmental company (the Japanese National Railways Settlement-of-account Enterprise) separate from the JR railway companies, has since risen to ¥27 million million, and interest payments on the debt alone amount to about

Australia plans high-speed train link

Sydney

AUSTRALIA'S plans for a Very Fast Train (VFT) link between Sydney and Melbourne have won the endorsement of Prime Minister Bob Hawke. In order to speed up the project, a Senate standing committee is to be established to examine the environmental, employment and development implications of VFT.

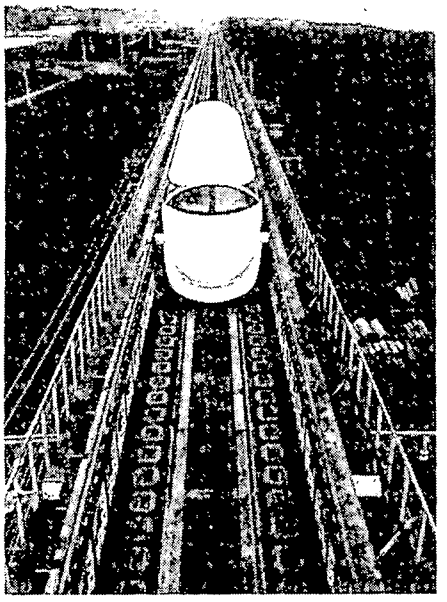
The train, described by Hawke as "the most important post-war development in Australia", will will travel at top speeds of 350 kilometres an hour, providing the fastest commercial train service in the world. The line should be in service by 1996 at a cost of A$4,500 million. Finance will come from private companies, including Broken Hill Pty Ltd, TNT, Elders IXL and the Japanese construction group Kumagai Gumi.

VFT will be important not just as a transport system but as a means of developing the Sydney–Melbourne corridor — a distance of about 1,000 kilometres, much of it relatively isolated. According to Alan Castleman, chief executive of the VFT Joint Venture, there will be several types of service. Twelve express trains a day will travel direct from Sydney to Melbourne in just over three hours. From city centre to city centre, the travel time will be the same as by taking an aeroplane. Twelve daily trains will stop at 14 towns between the two major cities as well as providing an express service to Canberra. Journey times between Sydney and Canberra will be cut from four hours to one hour.

According to Castleman, VFT will be travelling at 350 kilometres an hour for most of its route. The French TGV Atlantic presently travels from Paris to Lyon at peak speeds of 300 kilometres an hour, and the TGV Nord planned for 1993 will travel at 320–330 kilometres an hour on the way to the Channel tunnel. Whether the VFT can maintain such speeds routinely over distances much greater than in France is being questioned. Tania Ewing

Maglev track

¥1 million million a year. The powerful Ministry of Finance is thus strongly opposed to any plan that could place a further burden on Japanese taxpayers. The cost of a Tokyo–Osaka Maglev line is variously estimated at between ¥3 million million and ¥10 million million.



Maglev, up to 500 kilometres an hour? (Railway Technical Research Institute.)

Nevertheless, there is strong support among the ruling Liberal Democratic Party for building a Maglev line and more *Shinkansen*. JNR's debt has been quietly forgotten by most of the Japanese public and there is a saying among politicians that if you get a *Shinkansen* (or Maglev) station located in your constituency you are assured a place in the Diet for life.

David Swinbanks

SCIENTIFIC MISCONDUCT

Threats against witness

Washington

THREATS of legal action against a witness who testified at the most recent US congressional hearing on scientific misconduct have incurred the wrath of Congressman John Dingell, chairman of the House of Representatives subcommittee on oversight and investigations. As a staunch defender of the whistleblower in cases of misconduct, Dingell said he was "greatly concerned by what appear to be threats" and demanded evidence to justify them.

The incident was prompted by statements made by Dr Robert Sprague of the University of Illinois at Urbana-Champaign at a House of Representatives subcommittee hearing in June. Sprague, who exposed the fraudulent research of psychologist Stephen Breuning, said that the University of Pittsburgh tried to cover up the misconduct.

The vice-president and counsel for the medical and health care division of the University of Pittsburgh, George Huber, then wrote an angry letter to Sprague accusing him of making statements which were "not only inaccurate and untrue, but also slanderous and libelous". He said Sprague should stop making false statements about the university and should retract the inaccurate portions of his testimony, otherwise the university would take "whatever lawful corrective measures are necessary and appropriate, including the filing of legal action".

When Dingell heard of the letter, he responded with an equally angry letter to the president of the university, Wesley Posvar, expresssing his astonishment and saying he believed that Sprague's testimony was "substantially accurate". A week later, after a prompt review of the matter, Posvar informed Dingell that although there was no evidence of a cover-up, the university's investigation was not carried out with "sufficient zeal". He also wrote to Sprague, apologizing for the letter and commending him for his "vigilance and initiative on behalf of academic integrity".

Sprague says he criticized the university because its first three investigations focused only on research carried out by Breuning under subcontract to the University of Illinois and failed to scrutinize the research carried out for the University of Pittsburgh. No fraud was found in Breuning's research at the University of Pittsburgh until a fourth investigative committee reported on the matter in 1985, 18 months after Sprague's first accusations. By that time, Breuning had resigned, having admitted falsifying data related to his previous work with Sprague at the University of Illinois.

The university's counsel, Lewis Popper, who carried out the review, admits that it is "somewhat difficult to understand" why the university did not document and expose the fabrication of data at Pittsburgh and he says the university can be "legitimately criticized" for not doing so. Sprague's "impatience and ire" are understandable, he says. But he maintains that there is no evidence of an intentional cover-up. One reason why the committees might not have examined research at the University of Pittsburgh is that Sprague's initial allegations and proof focused on research carried out for the University of Illinois, says Popper. But Sprague says that the evidence supplied to the university was "equally devastating in both cases". For the investigations to ignore the research at the University of Pittsburgh was "mighty strange", he says. "I don't know how that could be explained by incompetence."

Popper also says that because Breuning had already resigned, it was difficult to investigate him and "arguably futile" to sanction him; that research was at an early stage and was terminated before he left; that the public health threat from fraudulent research at Pittsburgh was small compared with the risk from the research under subcontract to the University of Illinois, although Sprague disputes this; and that at that time there was less sensitivity and experience with investigating research misconduct that exists today. Because there is no evidence for a cover-up, Popper says, Sprague could be "legally vulnerable" if he repeated his accusations.

Sprague now says he will not repeat the allegations in future but will merely show his evidence and let others draw their own conclusions.

Christine McGourty

CONSERVATION

Biodiversity plan gets backing from NSF

Washington

LAST week, the US National Science Foundation (NSF) gave its backing to an ambitious plan to help preserve the Earth's biological diversity and halt what it calls "the most catastrophic loss of species in the last 65 million years". But the plan is likely to face an uphill struggle for funds in Congress.

In a report endorsed last week by the National Science Board entitled *Loss of biological diversity: a global crisis requiring international solutions*, NSF's committee on international science says that the foundation should increase funds for basic research in conservation biology, restoration ecology and environmental management, undertake an inventory of all living organisms, especially microorganisms, and increase support for research on biodiversity in developing countries.

To implement the plan would require in the first year a doubling of funds for biodiversity research, currently $20 million a year, followed by further annual increases until expenditure reached about $75 million. The 1990 budget includes a request for $30 million, but that is unlikely to be met in full.

NSF says that it should take the initiative in the study of global biodiversity because at present it provides the major part of federal support for systematics and ecology research in colleges and universities. The announcement of the new plan is likely to pre-empt attempts in Congress to set up a National Center for Biological Diversity and Environmental Research within the Smithsonian Institution.

Christine McGourty

ELECTRICITY GENERATION

Privatization makes progress

London

THE two new British electricity generating companies, which will take over from the Central Electricity Generating Board (CEGB) when the industry is privatized, were launched last week. Lord Marshall, chairman of National Power, the larger of the two companies, said they relished the challenges of free market and that it was "wonderful" to move away from government control.

National Power will take over the lion's share of CEGB assets in preparation for its flotation, including responsibility for Britain's nuclear power stations. PowerGen, the smaller company chaired by Robert Malpas, which was formed to create competition, will be responsible for 26 per cent of the electricity supply. A third company, the National Grid Company, will run the transmission network. This will be a multi-million pound concern owned jointly by 12 distribution companies.

Many questions remain to be answered about the timing of the £12,000–£13,000 million sell-off and the future of nuclear energy. Britain's ageing Magnox reactors have been withdrawn from the sale but National Power will still inherit 46 power stations, including five nuclear advanced gas-cooled reactors (AGRs), from the CEGB. Lord Marshall dismissed the suggestion that the AGRs would also be hived off and said the problems and costs of decommissioning them are "negligible" compared with Magnox. An intense dialogue continues, however, between the government and National Power about possible changes in future safety regulations. National Power maintains that it cannot be responsible for changes in regulations such as more rigorous safety procedures. The government has offered £1,000 million to pay for "unforeseen nuclear costs" and is to put an increased sum of £2,500 million before parliament this autumn to sweeten the nuclear pill.

The flotation timetable is in disarray following the decision to remove the 26 Magnox reactors from the sale because of the potentially crippling costs of decommissioning them by 2002. The two companies were to be sold separately in autumn 1990 and spring 1991 but now a six-month delay may result in a joint flotation shortly before the next election.

At a press conference in London, Andy Roe, National Power's information officer, said they were still committed to the introduction of pressurized water reactors (PWRs). The only problems lay in the immense capital expenditure and the need for a guaranteed market. It is essential for the National Power and the generation boards to draw up contracts to reduce the risks of building PWRs. He added that the PWRs were needed for National Power to reach the required production level of non-fossil fuel laid out in the privatization bill. He also said the obligatory 600 MW of renewable fuel would be produced by the wind farm in Capel Cynon, South Wales.

Mr John Collier, chairman of the UK Atomic Energy Authority, said the nuclear industry would suffer from the government privatization programme. He said it was "unfortunate" that the government would be cutting research into fast reactors from £50 million to £10 million and would end support for the prototype fast reactor at Dounreay by 1994. In the authority's annual report, he also said the drop in funding for nuclear fusion was "very serious". **Ben Webb**

ENVIRONMENT

Rain-forest canopy remains elusive ...

São Paulo

A NOVEL attempt to explore the upper canopy of the Amazon forest with a 'raft' suspended from a hot-air balloon was brought to a sudden halt last week when ten members of the French expediton were expelled from Brazil. The French group, headed by botanist Francis Hallé of the University of Montpellier II, had begun their activities without formal approval from the Brazilian government.

After a long wait for official approval of the project (see *Nature* **339**, 569; 22 June 1989), Hallé and his colleagues arrived in Brazil with only tourist visas. They then test-flew their 'flying raft' near Manaus, in the state of Amazonas, without permission, angering the Ministry of Aeronautics.

Approval had been granted for the scientific part of the expedition from the Special Secretary for Science and Technology and the National Council for Scientific and Technological Development. But under Brazilian law, final approval could be granted only after study of the expedition proposals by the military.

The technicians were rash to set up the dirigible without waiting for final approval, admitted Gérard Xavier Kuhn, science and technology attaché at the French Embassy in Brasilia. Although Brazil's Ministry of Foreign Affairs was trying to play down the affair last week, the researchers will have to wait outside the country for approval for the expedition and for the proper visas. **Ricardo Bonalume Neto**

. . . and there's no help from Washington

Washington

IN the United States, another novel project to reach the largely unexplored canopy of the tropical rain forest has ground to a temporary halt, but for a more conventional reason — the US Congress did not provide funds for it in the 1990 budget. Alan P. Smith, a scientist at the Smithsonian Institution, proposes to use a tall crane to reach the canopy. The crane could be set up even in inaccessible forest sites using a helicopter and, if required, moved from place to place. Precise and repeated access could be had to any point in the canopy within an 80-metre radius through the use of a manned gondola lowered from the crane.

Smith, who hopes to use the crane at the Smithsonian's research sites in Panama, says that the lack of such equipment means that carrying out research on the canopy is "like trying to do marine biology without a scuba system". Aerial ropeways have been constructed at several rain-forest sites but give easy access only to large branches, not to the branch tips where the flowers are, and where photosynthesis takes place.

A drawing of the crane, set in the midst of rain forest, appears in an exhibition at the Smithsonian which opened last week to celebrate the institution's tropical research and the completion of new research centre in Panama. But the crane itself, which requires an expenditure of $300,000 plus construction costs, will have to wait until Congress is in a more generous mood.

Alun Anderson

INSERM

Monkey dispute

Paris

WHILE members of the French anti-vivisection group, Arche de Noé, face charges of theft, the 28 macaque monkeys stolen from a Lyons laboratory of the French national institute of health and medical research (INSERM) in May have still not been handed back to their owners (see *Nature* **339**, 648; 1989). On Monday, INSERM's lawyer called for new charges of cruelty to be brought against the accused. Meanwhile a court of appeal was due last Tuesday, 22 August, to decide the fate of the monkeys, following the expiry of a custody order.

INSERM claims that identification tattoos removed by Arche de Noé involved the animals in unnecessary suffering and says that the wounds had not healed well. Arche de Noé has denied this. In a communiqué the group says that "the monkeys were operated on by a team of three veterinarians, under general anesthetic and in conditions of perfect asepsia".

"It is inconceivable", says the communiqué, "that our colleagues who risk their freedom for animals should be accused of acts of cruelty against these same animals." **Peter Coles**

SLC changes its tactics

San Francisco

AFTER a short-lived day in the sun during which it became the world's leading producer of elusive Z^0 particles, the Stanford Linear Collider (SLC) is now preparing to change tactics in order to avoid direct competition with a more powerful machine gearing up to speed at CERN, the 14-nation European scientific consortium based in Geneva.

For the SLC, which currently produces about 10 Z^0s a day, the change has come too soon. By studying the Z^0, one of the fundamental particles of the unified electroweak force, physicists will be able to pin down some of the poorly known parameters in their 'standard model' of elementary particles and forces. The original plan called for up to two years of dominance before the rival Large Electron–Positron (LEP) collider started up at CERN. Indeed, in the past few weeks, SLC scientists have announced the world's most accurate value for the mass of the Z^0, as well as a new limit on the number of 'families' — sets of related quarks and leptons — into which elementary particles are classified.

But nagging technical difficulties have put the collider far behind schedule and dramatically reduced its time at the top.

Earlier this month, the more powerful LEP made its first Z^0 particles (see *Nature* **340**, 495; 17 August 1989), signalling a new era for the SLC. Once the European collider concludes its pilot run and is refined for full-scale efforts around the end of September, its California counterpart will have to relinquish its leadership role and instead try to fill niches. "It just really depends on how quickly CERN works its bugs out. After approximately October 1, it's going to be difficult to compete on a particle-by-particle basis", said SLC spokesman Michael Riordan. "We have to do special things that the nature of our detector allows us to do."

The first tactic involves electron beams, which collide to produce the Z^0 particles. While the LEP beams come together at a much higher rate than SLC's, and consequently produce many times more particles, the California facility employs a narrower beam that offers its own advantages. Because the particles the Z^0 decays into are extremely short-lived — lasting on the order of a trillionth of a second — many of them will decay while still within the wider beam used at CERN. Consequently, the LEP physicists will not be able to tell which came directly from Z^0s and which are secondary creations, according to Riordan. The detection equipment needed to make such determinations should be installed at the SLC in early October.

For the longer term, beginning perhaps in 1990, the SLC will use polarized electron beams, in which the electron spins are aligned with respect to the beam axis. By giving SLC physicists an extra degree of control over reactions that make Z^0s, polarized beams permit the same level of accuracy to be achieved on certain measurements with 10 times fewer Z^0s than CERN. In measuring the Weinberg angle, the basic parameter of electroweak unification, SLC and CERN can thus compete on even terms.

In recent weeks, news accounts have focused on the well-known competition between the two facilities. By switching tactics, the SLC group hopes to play down the rivalry with CERN and emphasize ways for the two efforts to complement each other. **Robert Buderi**

EDUCATION REFORM

UK university charters stay the axe

London

THE resolution last week of a dispute over academic tenure at Aston University in Birmingham indicates that half of Britain's universities are still unable to dismiss tenured staff almost one year after the new education reform act ruled that they could.

Aston University wanted to impose compulsory retirement on 12 members of staff in the department of engineering. In response, the Association of University Teachers (AUT) threatened to take out an injunction to prevent the dismissals. The ensuing legal battle ended when the vice-chancellor of the supreme court, Nicholas Browne-Wilkinson, who was appointed to resolve the dispute, ruled that Aston's charter and statutes protect academic staff from compulsory redundancy unless the university has "good cause" to dismiss them. More than half of Britain's universities have similar charters.

Many universities are attempting to reduce staffing levels by voluntary redundancies and retirements to compensate for financial cutbacks and falling student numbers in certain fields. But some universities are being forced to try more drastic steps to reduce costs. The new Education Reform Act allows the dismissal of tenured staff, but the charters under which the universities operate must first be rewritten. Five senior academics and lawyers have been asked by the Department of Education to look at individual charters to amend the clauses that guarantee tenure.

The new legislation is not retrospective, however, and any academic appointed or promoted before 20 November 1987, when the bill was published, will still be protected. David Packham, Aston's secretary and registrar, said that this was a serious flaw in the legislation and that some universities might suffer serious financial problems because they are unable to shed the academic posts they cannot afford. "The answer for some may be bankruptcy to save themselves and to worry about the consequences later," he said.

Ben Webb

SATELLITES

Indian launch vehicle grounded

New Delhi

INDIA'S Augmented Satellite Launch Vehicle (ASIV) has been grounded on the advice of an expert review panel after two successive failures. The panel has asked the Indian Space Research Organisation (ISRO) to modify the design of the rocket before attempting another flight. According to ISRO, the third ASLV flight incorporating the suggested changes will not take place for a year or more.

The panel suggested the design changes after a detailed investigation of the crash of ASLV during its second flight in July 1988. It concluded that ASLV is highly unstable (because of its high length-to-diameter ratio of 23) and that its control system is inadequate, particularly during its flight in the lower atmosphere where dynamic pressure is high. No control of the rocket is available during the critical phase between the burn-out of the two strap-on booster motors and the ignition of the first-stage motor. During the abortive ASLV flight, the boosters burnt out a little too soon and the period of "no control" lasted for four seconds, during which the yaw angle rapidly increased. The increased load caused the vehicle to break up. These design inadequacies were not revealed in the first flight on 24 March 1987, as its failure was due to non-ignition of the first-stage motor.

ISRO has been asked to redesign the auto-pilot system and strap-on boosters, enhance the control margin and confirm them through additional simulation and destructive structural tests. All this work will be done by a new interdisciplinary design group. ISRO has admitted that the original design of ASLV failed to recognize the full impact of inadequate control during the critical phase flight or that of wind shears and gusts on the structural margins of safety.

ASLV is expected to be ISRO's workhorse for placing payloads up to 150 kilograms. But lessons learnt from it have already led to design modifications in the Polar Satellite Launch Vehicle (PSLV) which will place one-tonne payloads in polar orbit. **K. S. Jayaraman**

Peer review (continued)

SIR—It is time that government funding agencies provided grants in support of the peer-review system. This crucial component of the scientific process can no longer be left to the goodwill of individuals because pressure of work means that reviewing has a low priority.

In Darwin's time, peer reviewing was easy. Most studies were descriptive or involved simple experiments. Today, reviewers and editors are no longer able to verify an experiment on a desk top because experiments are costly and time-consuming. For the same reasons, accurate and detailed communication of results by publication has become increasingly important.

At the same time, the pressures on individual scientists have caused the review process to become slower and less thorough. Prominent scientists may get 50 to 100 papers a year to review from colleagues and from journals. Some recent papers in mathematics have exceeded 100 pages and the effort required to review such a work exceeds the time available to most busy scientists.

My proposal is to provide for a rotating group of paid reviewers. Grants would be given for three months of work as a reviewer. They would be given on a lottery basis to individuals with a reasonable publication record, with the number of grants given proportional to the number of publications in each field. Individuals would be paid for their time and expenses. Each person could get the grant only once in, say, five years to avoid concentrating too much influence in a few hands. A listing of reviewers' specialities would be put into a database from which journals could pick those to whom they would send papers for review. As a limited supply of reviewers would be available because of limitations on funding, journals would be best served by sending to this group of paid reviewers only papers requiring extra attention, such as very long, very difficult or controversial papers. Most papers would still have to go to volunteers. But if the volume of papers needing review was reduced by this method, then volunteer reviewers might be able to do a more thorough job on the shorter or less controversial papers.

We have accepted the fact that many special tasks must be paid for in science. Whereas time used to be given voluntarily for editing scientific journals, most now have paid editorial assistants and even editors. Statisticians and other experts are paid for their advice and National Science Foundation grant-review panels are funded. Why do we not see reviewing as a form of consultation? Only by paying for someone's time — and thus providing recognition for their work — are we likely to increase the level of effort people are willing to put into reviews.

A final consideration concerns the purpose of the paid review. There are two types of reviewer, the gatekeeper and the mentor. The gatekeeper tries to find fault and reject a substantial number of papers, in the name of professional standards, and this can be done quite quickly. If someone is being paid, he or she can perhaps take the time to act more as a mentor. In this capacity, a reviewer would check the equations, assist the author with clarity or logic, and spend some time really thinking about the paper. Such careful review may require reading related papers cited by the authors or performing some calculations. But if a study takes several months or a year to carry out and write up, a careful review should take a day or two. A mentor-type reviewer who does the job well may even end up as a coauthor on the paper, an added benefit of the position.

The question of funding for this system of grants of course arises. In fact, very little extra money would be required. Reviews are at present a 'hidden overhead', conducted in time paid for by grants given for other purposes. The costs of not having paid reviewers are very high. Papers are delayed for long periods waiting for reviews, and inadequate reviews do not help the authors to revise the paper for resubmission. Poorly written papers do not advance the field, and errors not detected before publication cause confusion and lower the reputation of science as a whole.

If 2,000 grants were awarded each year for three months of reviewing, then up to 200,000 papers could be reviewed thoroughly (assuming one to two days per review) at a cost of only $30 million (salary plus overhead). The only cost is for direct labour, whereas normal research budgets include large amounts for expensive laboratory equipment and supplies. Spread over the huge budgets of the government funding agencies, this figure is a drop in the bucket.

CRAIG LOEHLE
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SIR—Although anonymous peer-reviewing has produced excellent results, the system has not been immune to insults and abuse (for example Craig Packer, *Nature* **340,** 10; 1989). Here is a simple protocol that could bring fresh hope and restore the respect the system deserves. I suggest that from now on the authors become anonymous and the reviewers come out of hiding. In this system, a journal editor would simply send a manuscript to a potential reviewer in the usual way but omitting the author's identity. The reviewer would have the option of remaining anonymous or having his or her identity revealed. Authors' identities should ideally have no bearing on the evaluation of a scientific piece of work.

GOBINDA SARKAR
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SIR—It is an indictment of the scientific community if one of us could even be suspected of being remotely instrumental in the precipitous action taken by Craig Packer's colleague (*Nature* **340,** 10; 1989). In practice, most editors maintain the anonymity of all reviewers by distributing unidentifiable comments to authors and fellow reviewers. An equally fair system would evolve if anonymous manuscripts were peer-reviewed. Editors can request that copies of submissions for this purpose should have their title on the abstract/summary page without details of the author(s) or institution(s). The onus would then be on contributors to write their papers in a style that would make their ready indentification less easy. One would then hope that personal attacks, most of which may indeed never come to light, would be eradicated at once.

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Names please

SIR—It has been decided to do a volume of omissions from the *Dictionary of National Biography* from the beginnings to 1985 (the 1981–85 supplement will be published next spring).

The new volume gives people an opportunity to suggest names of those they have looked up in the *DNB* and not found. This is the first time for a hundred years that such an opportunity has occurred. Previous volumes of the *DNB* have not been as generous to scientists as they have been to people from other walks of life. I would therefore be glad to hear from any of your readers with names to suggest.

C.S. NICHOLLS
Dictionary of National Biography, Oxford University Press, Clarendon Building, Bodleian Library, Oxford OX1 3BG, UK

Correction

The author of the letter entitled "India's too costly imports" (*Nature* **340,** 94; 1989) is Y.D. Sharma. □

Seeing the worst side of science

Andrew Tudor

Since the advent of the genre in the 1930s, horror movies have reflected public anxieties about science and technology. Through the years the images have changed.

"THE human mind will only stand so much — we're all a bit strange up here." So observes the aged butler in *Dr X*, First National's 1931 response to the huge horror-movie success of that year, *Frankenstein*. He was, of course, speaking of the dedicated band of scientists for whom he worked, their laboratory an isolated old mansion, their goal the creation of synthetic flesh. But he could as well have been describing horror-movie scientists at any time during the past 50 years, for, in this movie-made world, science is nearly always 'mad science'.

Along with supernature and psychiatric disorder, mad science is one of the three most common sources of horror-movie monsters. Of the horror movies made between 1931 and 1984 that I have researched (almost 1,000 of them), more than a quarter posit science as one of the main causes of disaster. In so doing they tell us, if through a glass darkly, something about what people have found frightening over the years. In Tuan's elegant phrase, they map for us our "landscapes of fear", providing a fascinating insight into changing popular images of what is threatening about science and scientists. After all, if they are to make sense to their audience, they have to accord at some level with that audience's attitudes, prejudices and fears.

When you examine the genre's history, one general trend is immediately apparent. As the figure (overleaf) illustrates, there is a broad decline in the proportion of science-based horror movies after 1960, a decline coincident with the modern rise of the horror-movie psychotic. In the popular consciousness of the 1970s and 1980s it would seem, mad science has given way to a different kind of madness. Look more closely at the history, and it is also apparent that there are some periods when the threat posed by individual mad scientists is less important than that posed by science itself. This is so in the 1950s and in the late 1970s and 1980s, reflecting the different evaluations of scientists' responsibility typically found in the movies of these years. With the growing public concern about atomic energy during the 1950s, the idea that scientists might unwittingly cause disaster becomes more common, a pattern that is repeated 20 years later when scientific side-effects are seen to give rise to pollution and ecological imbalance.

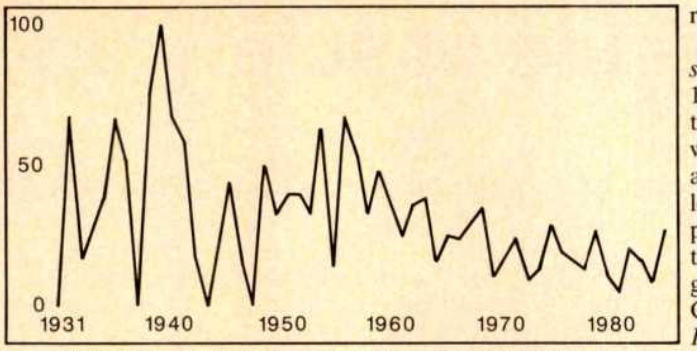
This issue of scientists' personal responsibility is central to the shifting pattern of the horror-movie vision of science. In the 1930s and 1940s, the actions of individual mad scientists were all-important — whether they were evil men pursuing their own ends or good men corrupted by their overwhelming commitment to science. In the 1950s that changes; although it is still scientific knowledge that routinely causes mayhem, scientists themselves are more often portrayed as our saviours than as our executioners. Then, as the threat of horror-movie science recedes during the 1960s and 1970s, science is assimilated into a general fear of big institutions — multinational corporations, the state, the military — exploiters of scientific research who are represented as ruthlessly destroying our environment for the sake of profit and power.

British Film Institute

A character with "higher aspirations" — Dr von Niemann in *The Vampire Bat* (1933) seeks to create life in the laboratory but is corrupted by his obsession.

One fictional figure towers above all others in the classical development of horror-movie science. Baron Frankenstein, introduced in the spoken preface to the 1931 film as "a man of science who sought to create a man after his own image, without reckoning upon God", is the archetypal mad scientist. Devoted to the pursuit of knowledge at the expense of humane values, he and his successors (whether or not they bear his name) are permitted the equivocal comfort of defensible scientific motives. "Where should we be if nobody tried to find out what lies beyond?", asks Henry Frankenstein in *Frankenstein*, "Have you never wanted to look beyond the clouds and stars, to know what causes trees to bud and what changes darkness into light? But if you talk like that people call you crazy." Indeed.

At the other end of the decade in the exquisitely designed *Son of Frankenstein* (1939), where it rapidly becomes clear that the new Baron (played by Basil Rathbone) has inherited more than just an aquiline nose from his infamous father, the generic proclamation of scientific commitment comes in a letter that father has left for him: "If you, like me, burn with the irresistible desire to penetrate



Percentage of threats in horror movies caused by science.

the unknown, carry on. Even though the path is cruel and tortuous, carry on. Like every seeker after truth you will be hated, blasphemed, and condemned". Frankenstein, inspired by these words from the past and later shown his father's tomb on which someone has chalked "maker of monsters", takes up a symbolically burning torch and, using it as a pen, alters the crude epitaph to read "maker of men".

Such characters have higher aspirations (as one might guess from the way they so often fix their eyes on some distant off-screen point). Although most narratives do contrive to demonstrate that they are corrupted by their obsessive pursuit of knowledge, many movies also allow that relations between scientific and human interests may be genuinely problematic. Even someone as finally unredeemable as Dr von Niemann in *The Vampire Bat* (1933) is permitted an impassioned speech about the glories of science. "Mad?", he says, "Is one who has solved the secret of life to be considered mad? Life, created in the laboratory. No mere crystalline growth, but tissue, living, growing tissue that moves, pulsates, and demands food . . . . Think of it. I have lifted the veil. I have created life. Wrested the secret of life from life."

Of course, the fact that von Niemann finally comes to a sticky end (shot by his vengeful assistant) demonstrates his obsession to be truly insane. Yet the line dividing movie madness from genius is often less than clear; science, however threatening, also has its potential for good. So although existing authorities, such as the police or the local burghers, are the main narrative resource for dealing with those who stray from the path of scientific propriety, many of the period's monsters and their creators are finally destroyed not by those authorities but by each other, as if in implicit recognition of their joint sins. Only through self-sacrifice can they be redeemed.

In the *Frankenstein* movies of the 1930s, sympathy is therefore as much with the Baron and with Karloff's anthropomorphic Creature as with the official forces of good. When the Creature in *Son of Frankenstein* studies his unprepossessing reflection in the mirror, drawing Wolf von Frankenstein next to him to better make the comparison, we are more touched by his plight than by that of his erstwhile victims. And when the storm first ignites life in that lumbering frame in *Frankenstein*, it is the Baron's staring intensity at the scene's end which attracts all our involvement. "It's alive", he mutters, caught up, as we are, in the enormity of his achievement.

Thus it is that most of the mad-scientist movies of the early years, including *Dr Jekyll and Mr Hyde* (1931), *Dr X*, *Murders in the Rue Morgue* (1932) and *Frankenstein* itself, express at least qualified admiration for scientific inquiry. Fear of science is not straightforward here, for it is tempered by nascent ideas about the need for progress and the price of that need. As they struggle to create life, whether in the travesty humans of the *Frankenstein* tradition, or through the mechanical hearts of *The Walking Dead* (1936) and *The Man They Could Not Hang* (1939), or even in the ape-humans of *The Monster and the Girl* (1941) and *Dr Renault's Secret* (1946), these mad scientists reflect both the terror and the promise of science.

In the final analysis, though, horror-movie science of this period *is* fundamentally disordering, revolving around inhuman ambitions which, by their very nature, give rise to inhuman threats. In seeking to interfere with the basic processes of life, science trespasses in areas quite properly forbidden to it — a belief occasionally given crude religious justification, though more often simply taken for granted. Dazzled and corrupted by the prospect of knowledge, scientists ignore the limitations implied by everyday values and loose their misconceived beasts upon an unsuspecting world. In this view scientists are powerfully threatening, their capacity to manipulate nature undoubted and all-embracing. As one character observes in *The Electric Man* (1941), "this theory of yours isn't science — it's black magic". Faced with such power, it is only by resort to violence and coercion that order can be restored and the pre-science world once more made secure.

In the 1930s and 1940s, then, the heyday of mad-science movies, scientists were either corrupted by their commitment or, less interestingly, they were evil men who bent science to satisfy their personal desires. But in the 1950s a new conceptualization of science-as-threat emerges. It had its precursors, of course. When the scientist of *The Invisible Ray* (1936), played by Boris Karloff, was driven insane and finally destroyed by Radium X, it was the first significant horror-movie allusion to the unexpected dangers of nuclear science. Twenty years later, with the likes of the giant ants of *Them!* (1954), the unseen radiation-consuming creature of *X the Unknown* (1956), the prehistoric monster liberated by nuclear explosion in

Objects of pity — Boris Karloff's anthropomorphic Creature in *Frankenstein* (1931), and his creator, the Baron, inspire as much sympathy as do the official forces of good ranged against them.

Godzilla (1957), and the mutated molluscs of *The Monster that Challenged the World* (1957), the deliberate creations of the mad scientists of the 1930s have given way to the accidental products of nuclear testing.

There are two main aspects to this change. One derives from growing public awareness of the radiation risks of atomic energy. The other — though not unconnected — is more complex, extending and generalizing traditional horror-movie ambivalence about scientists' responsibility for their actions. In the 1950s scientists are no longer simply mad or bad — although they are dangerous to know; rather they have become the necessary instruments of progress. There *is* fear here, of course, the generalized fear that the engine of change is out of control, progress is not an unqualified force for good, and individual scientists — however well intentioned — are neither responsible for, nor in command of, the outcome of their researches.

Such a fear is central to the many films of the 1950s that focus on the monstrous side-effects of atomic energy. But, interestingly, it also permeates some of the period's more traditionally framed horror movies. Take *The Fly* (1958), for instance, one of the most commercially successful mad-science movies of the decade, and the first to be produced by a major studio (Fox) in both colour and CinemaScope. *The Fly* presupposes the familiar stereotype of the obsessed scientist whose precipitate haste to complete his research brings disaster. In the event, he is the victim. Testing his matter-transfer apparatus on himself, he unwittingly enters the machine in company with a fly, emerging with the fly's head and one claw-like hand, his own head and arm imposed on the insect. With no hope of reversing the process, he destroys his notes and his equipment, persuades his wife to cooperate in crushing his body in an industrial press, and leaves his brother to restore familial order with his wife and child.

This is a clear enough variation on the Frankenstein model. Yet where the scientist, André, differs from so many of his predecessors is in the absolute sympathy that the film accords him. He could hardly be described as 'mad' even to the limited extent of, say, Wolf in *Son of Frankenstein*, and his disastrous haste is never motivated by a desire for self-aggrandizement. It is, rather, based on his wish to help humanity by exploiting the positive potential of his discovery. Unlike traditional mad scientists, he is not to blame; like all of us he is a victim of the modern age, turned into a creature of pathos as much as one of horror.

Thus, although the price of progress is certainly high in the films of these years, individual scientists are often relatively blameless. After all, this is the era in which the 'scientific expert' is most effective in dealing with a wide variety of movie threats, not simply those created by science itself. Specialist scientific knowledge is recognized as both dangerous and essential, scientists' expertise irreplaceable: Professor Quatermass may be indirectly responsible for the invading space monster of *The Quatermass Experiment* (1955), but he is also the only person who can destroy it. This double-edged evaluation of scientific expertise was not new, of course. But it was given additional force in the 1950s, perhaps most clearly in those radiation-obsessed horror movies whose ancestor was *The Invisible Ray* of 1936. There is an irony here, a definite discrepancy between the enormity of the nuclear threat and the credibility routinely afforded to 1950s' science. Perhaps, as so many have argued, our popular culture really was trying to teach us "to stop worrying and love the Bomb".

Many films of the 1950s feature scientists whose pursuit of scientific goals is admirably motivated. Deemer, for example, the biochemist in *Tarantula* (1956), is researching nutrients in the hope of resolving the world's food problems, work which kills him and his staff as well as releasing an overgrown spider into the local community. Typically, only a combination of scientific expertise and military strength finally eliminates the threat (the hapless arachnid is finished off with napalm). Such films are notable in that their scientific researchers are neither obsessed nor evil; they are simply unlucky. In trying to improve the lot of humanity they have the misfortune to destroy themselves or others. In this movie world, science no longer corrupts its practitioners; but it is, by its very nature, a risky enterprise, and for that reason it can often be dangerous.

British Film Institute

An early allusion to the dangers of nuclear science — Boris Karloff (left) plays a scientist driven insane and finally destroyed by Radium X in *The Invisible Ray* (1936).

Unsurprisingly, the Frankenstein tradition of crazed and corrupted scientists does not sit easily in the horror-movie world of the 1950s. There are some mad scientists, but they are often more humorous than horrific. Just the title of *I Was a Teenage Frankenstein* (1957) suggests the lower depths of invention, although it does boast an immortal line delivered to his teenage creation by the film's mad medico: "I know you've got a civil tongue in your head because I sewed it there myself". Only Hammer Films (beginning with *The Curse of Frankenstein* in 1957 and continuing throughout the 1960s) really sought to extend the traditional Frankenstein conception. In so doing, they shifted its emphasis away from the corrupting potential of science and towards a more simplistically evil characterization of the mad scientist.

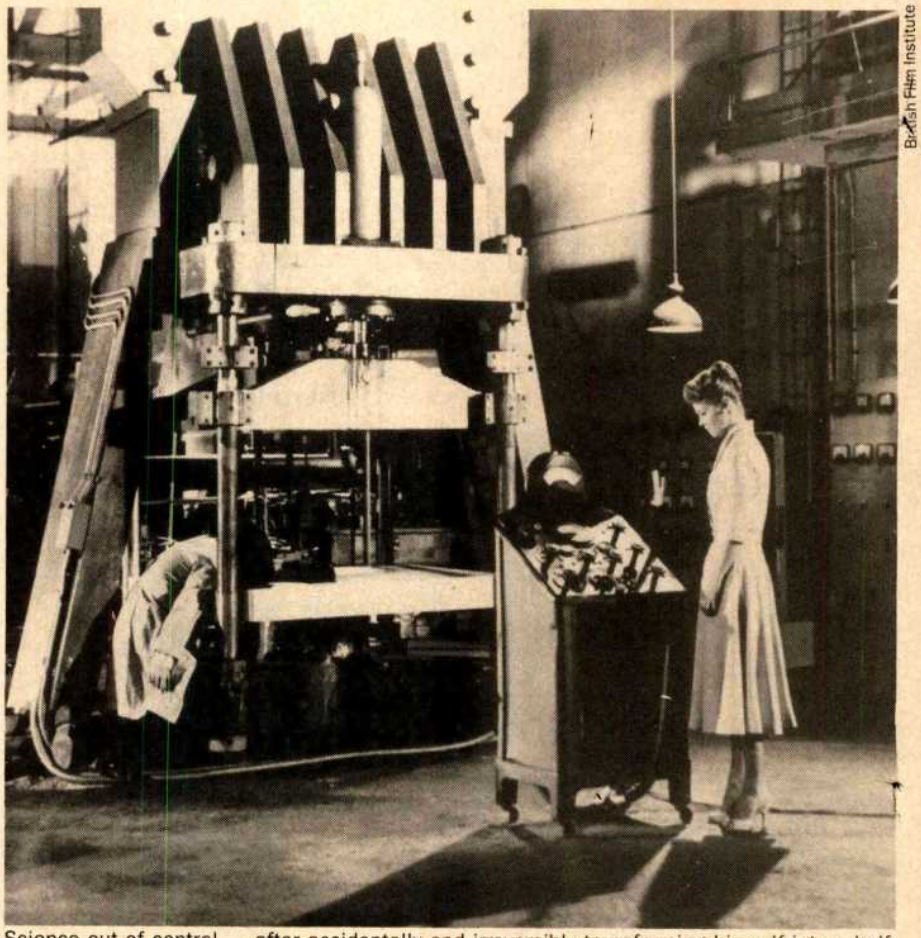
In the 1950s, the inhuman ambitions of individual scientists are far less to the fore than hitherto, and the familiar moral ambiguity of the Frankenstein tradition is painted onto a much larger canvas. This science is no longer the quasi-magical activity of the 1930s, tucked away in old houses and gothic castles; it is more prosaic and more all-embracing. Science has become a constitutive part of our everyday world, its admired and feared exponents harbingers of both progress and disaster, its most common threat — radiation — unseen, but a potential invader of every corner of our lives. Perhaps, then, the most symptomatic image of this period is not Godzilla laying waste to Tokyo or

the giant ants of *Them!* struggling for their lives in a city sewer. It is the eponymous hero of *The Incredible Shrinking Man* (1957), dwindling victim of accidental irradiation, desperately seeking only to survive in a universe which no longer offers him (or us) a secure and central place.

As this assimilation of science into the everyday world extends through the next three decades, scientific research is increasingly represented as no more than a variously effective means for pursuing variously lunatic ends. Whether one seeks to rule the world by reanimating refrigerated Nazi soldiers, as in *The Frozen Dead* (1967), or merely to capture the heroine's heart by mutating into a jellyfish and killing off the romantic opposition, as in *Sting of Death* (1968), science largely serves as an unexplicated instrument of evil. Although many favourite mad-science enterprises still remain credible — solving the secret of life, preserving youth, fostering mutations — the concept of science to which they relate has lost whatever distinctiveness it once had. Science in itself is no longer intrinsically frightening, and scientists, whichever side they are on, are much diminished. Faith in the expert scientist as a bastion against all manner of threats has virtually disappeared by the 1970s, a decline which is, finally, the most characteristic feature of this last phase in the evolution of horror-movie science. By the late 1970s, even the most explicitly science-orientated of horror movies actually pay little attention to science.

Thus, although science may be necessary to precipitate the kind of ecological disasters common in modern horror movies, as in *The Crazies* (1978), where a biological weapon is accidentally released into a town's water supply, it is more often the military, the state or the big corporations who are seen to be guilty. What was once a consequence of scientific over-ambition or individual misuse of knowledge is now integral to the activities of the very authorities who used to be central to our defence. This presumed conspiracy between the institutions of science, state, military and industry is a thread running through many modern horror movies, not simply those which make overt reference to science. In *Piranha* (1978) the rampaging fish have been created (by science) as weapons for use in Vietnam. Accidentally released into a local waterway, their existence the subject of a military cover-up, they can only be destroyed by polluting the water with chemical waste from a convenient factory. If, that is, they are destroyed — for, like most such films, *Piranha* keeps its options open.

Generally, of course, horror-movie pollution has the opposite effect. The spreading mutations of *Prophecy* (1979) are a consequence of mercury poisoning from a local lumber mill; the unnatural nasties of *Humanoids from the Deep* (1980) are the accidental product of experiments to increase the yield of local salmon; and the giant reptile of *Alligator* (1982) — of which its writer, John Sayles, revealingly observes, "my original idea was that the alligator eats its way through the whole socio-economic system" — mutates as a result of consuming animal remains from a big corporation's hormone experiments.



Science out of control — after accidentally and irreversibly transforming himself into a half insect/half human monster in *The Fly* (1958), the scientist persuades his wife to end the horror by crushing his body in an industrial press.

Science functions strictly instrumentally in modern horror movies. If mad-scientist films in the 1930s were about knowledge and its dangers, those of the 1980s are about something else. They postulate a world under threat of imminent destruction, individuals open to horrifying metamorphoses, and established authorities either helpless in the face of catastrophe or corruptly involved in its genesis. Science here is just one way in which power can be exerted over a desperately resisting population; scientists just one repressive tool among many. Science has become the servant of other interests, and the once visionary faith of the mad scientist has now been reduced to a fast-crumbling defence against apocalyptic social and physical collapse.

Perhaps it is not surprising. In a fictional world in which people can be routinely and inexplicably transformed into psychotics or zombies, a belief in science seems peculiarly inappropriate. Throughout the modern genre, expertise — meat and drink to science-based horror movies — has been devalued. As civilization collapses all around them in *Dawn of the Dead* (1980), a TV interviewer talks to a bearded and black-eye-patched scientist, an echo, perhaps, of Herman Kahn and the days of "thinking the unthinkable". The scientist, despairing and exhausted in the face of an intractable situation, still asserts the faith of experts everywhere. "We've got to remain rational", he says, "logical, logical, logical . . .". He sinks into hypnotic repetition of "logical" and the interviewer's voice rises above his: "Scientists always think in those kinda' terms. It doesn't work that way. That's not how people really are".

As ever in the horror movie, the scientist doesn't even hear him: ". . . logical", he continues, "we have no choice. It has to be that way. It's that, or the end". It is one measure of the pessimism of the horror movie's modern vision of science that "the end" is exactly what it turns out to be.

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High-energy physics needs thought

Proper excitement that CERN may answer many important questions should be moderated by some deliberate plan for true global collaboration in high-energy physics.

Big-science people are a patient lot, and have to be. Projects costing $1,000 million (such as the Hubble Space Telescope) or more (the Superconducting Super Collider, SSC, now to be built in Texas, for example) are necessarily years in gestation. Those who conceive of grand projects may often have died or retired by the time their dreams come true. The difficulties for younger people seeking to make their way in a competitive world on the basis of published contributions are even more acute. Often there may be nothing to give to prospective employers, or fellowship committees, except letters of recommendation from more senior people.

Much has been said about the sociology of these circumstances. That the originators of projects may not be those who see them through, and who reap the rewards of discovery, engenders a sense of teamwork and a seemly sense that discovery is (in Vannevar Bush's evocative phrase) an endless frontier. The minuses are different. The teams are often huge — so large that young people may be lost in them, or may find that success hangs on qualities not strictly those of science, managerial skill, for example. The other side of that coin is that young people also learn a great deal when working as members of large big-science teams.

There is also a sense in which large projects engender impatience. When people know that it will take a decade to complete a project, they are understandably eager to get on with it. But megaprojects almost always require that some government or governments should have learned to temper impatience with a proper sense of responsibility, persuading politicians that a project for which funds are sought is a significant improvement on what exists already, and is likely to yield a substantial harvest of discovery.

These platitudes are prompted by the appearance in *Physical Review Letters* of two articles describing the accumulated measurements at Fermilab and SLAC (the Stanford Linear Accelerator Center) of Z^0 bosons, likely in the next few months to be produced in great abundance at CERN (the European High-Energy Physics Laboratory at Geneva). It makes sense that the data should be published now, if only as a benchmark against which CERN can calibrate its own measurements. For what it is worth, the measurements described by Fermilab and SLAC (**63**, 720 and 724 respectively; 1989) agree within the experimental uncertainties both with earlier data from CERN and with the prediction of the standard theory due to Salam and Weinberg. Each article is signed by roughly 200 people; those interested may count them.

The SLAC article, interestingly, does not include the name of W.F. Panovsky, director at SLAC for many years until his retirement three years ago. That illustrates the selflessness with which the originators of important projects create them for the benefit of others: Panovsky was the driving force behind the creation at SLAC of the machine called the Stanford Linear Collider (SLC) with which SLAC's Z^0 bosons have been made. The idea is that pulses of electrons and positrons from SLAC's main electron accelerator are separated magnetically, then focused by means of magnets in roughly semicircular arcs about a kilometre in circumference and finally made to collide.

The technique is among the most exacting so far practised even in high-energy physics. Each pulse of electrons consists of 10,000 million particles. The trick is to arrange that each pair of pulses hits the same patch of geometrical space, roughly 3 micrometres in diameter, at virtually the same time. It is understandable that it should have taken a little longer than first planned — two years extra, by some estimates — to get the counter-circulating pulses into the planned shape. No doubt everybody working on the project has learned a great deal from the experience, the virtues of patience particularly. As the only particle accelerator in which bunches of particles are fired at each other as if they were bullets, SLC will also provide useful design experience for machines not yet built. The Soviet Union has already decided in principle to build such a machine at Serpukhov, while another on the same lines is spoken of as a successor to the advanced version of the Large Electron–Positron collider now producing Z^0 particles at CERN.

But there is a delicate irony in all this. If SLC had indeed functioned as designed when first commissioned, it would by now have generated some thousands of unambiguously identified Z^0 particles and CERN would have been deprived of the opportunity to produce the definitive data on the properties of these particles, now expected in the next few months. That does not mean that the money spent on LEP would have been wasted — there are plenty of other things to do with LEP (see *Nature* **340**, 277; 1989). But the gilt would have gone from the gingerbread. And while it would be wrong to say that SLC was built to steal CERN's thunder, the sense that this might happen helped to fortify the resolve of SLAC — and helped to persuade the US Congress to approve the money.

There is also a moral. Nobody will deny that competition has a place in science, or that the competition to build more effective accelerators and to commission them on time has been an efficient spur to the development of high-energy physics. But with even (perhaps, given the federal deficit, one should say "especially") the US Congress sucking its teeth over the prospective cost of SSC, the time has come to ask what can be done to make competition more efficient, or less wasteful of resources. There is, in other words, a need that the development of new machines should be invested with a still greater sense of deliberation, however painful the present slow process of winning approval for a new project may be.

This, sadly, is where the high-energy physics communities on both sides of the Atlantic become evasive. The obvious end-point in the field is global international collaboration. So much seems generally to be accepted by high-energy physicists in the United States and Europe, but with a reservation reminiscent of that applied by St Augustine to his prayer that God should make him chaste — "but not yet". The accelerator after next is when the time will be ripe, is what the people say.

That cannot continue to be a respectable riposte. Even as things are, the reality of transatlantic collaboration in the field is substantial, as is that between Japan and European and US laboratories. Why not formalize these arrangements, which would require some kind of general consensus about the design of the next generation of accelerators and agreement about their siting? People's reservations seem usually to be stimulated by the calculation that, then, there would be only one machine being built at any time. That need not necessarily be the case, and only one machine would be better than none, the outcome if the world's taxpayers turn sour. That might mean that patience would have to be more widely practised. Would that matter?

John Maddox

SYNAPTIC TRANSMISSION

Broad minded on narrow spikes

Robert Meech

IT IS natural to suppose that the longer and larger the action potential that arrives at a chemical synapse, the greater the rise in intracellular Ca^{2+} and the more neurotransmitter that it causes to be released. Just such a mechanism may contribute to the analgesic action of opioids in vertebrates[1] and to behavioural sensitization in *Aplysia*[2]. But on page 636 of this issue[3], A. N. Spencer, J. Przysiezniak and J. Acosta-Urquidi report that at the neuromuscular junction of the jellyfish *Polyorchis penicillatus* precisely the reverse is true: short action potentials produce a larger postsynaptic event than longer ones. This is not a specialization of simple systems like jellyfish, for the explanation is a general one with implications for nervous systems as complex as those of the insects or mammals.

In 1967, Katz and Miledi[4] used squid axon giant synapses treated with tetrodotoxin to show that transmitter release depends on the amplitude and duration of the depolarization at the presynaptic terminal. In depolarized squid axons, calcium activation develops so slowly that the rise in internal Ca^{2+} follows much the same course as the increase in potassium conductance[5]. Hence, when Llinás, Steinberg and Walton[6] reconstructed the events during normal synaptic transmission, they showed that the calcium responsible for transmitter release enters the terminal during the repolarizing phase of the action potential. Accordingly, the slower the rate of repolarization, the greater the calcium influx at the presynaptic terminal and the larger the associated postsynaptic response. This has been confirmed experimentally in vertebrates by Lin and Faber[7] using synapses on the Mauthner cell of the goldfish.

Prolonged action potentials are not always associated with a greater rise in intracellular Ca^{2+}, however. In molluscan neurons, successive impulses in a train of action potentials grow in duration as a result of the cumulative depression of voltage-dependent potassium currents[8]. Use of the calcium-sensitive dye arsenazo III injected into the giant neurons of *Aplysia* has shown that the action potentials may broaden by as much as 50 per cent with no apparent change in calcium influx[9]. This is because the pronounced shoulder on the falling phase of each of the prolonged action potentials is near 0 mV. Most calcium channels are closed at this voltage in these cells and so the prolongation has little effect on the change in intracellular Ca^{2+} measured by arsenazo.

Compared with *Aplysia*, the position of the calcium activation curve in squid[10] and *Polyorchis*[3] is shifted along the voltage axis so that there is a substantial inward current at 0 mV; this is illustrated for the *Polyorchis* motor neuron, (*a* in the figure) which shows a maximum inward current at about +10 mV. As a result, prolongation is most effective with relatively low amplitude action potentials. This is because the driving force on the calcium ion decreases as the membrane potential approaches the calcium equilibrium potential and, although the calcium channels are open, calcium entry becomes smaller not greater. With a large amplitude action potential it is not until the membrane begins to repolarize that the driving force becomes sufficient to promote calcium entry.

The *Polyorchis* preparation differs from the squid synapse in that its action potential is sufficiently prolonged for calcium channel inactivation to become significant. In *Polyorchis*, the entire overshoot of the shorter action potential is associated with a near maximum inward calcium current (*b* in the figure) but the peak of the longer action potential is out of this range (*c* in the figure) and so there is relatively little calcium entry until the later stages of repolarization. Spencer *et al.* suggest that during the early stages of the action potential many calcium channels are inactivated and become unavailable. As a result, calcium enters the cytoplasm at a low rate and much of it is sequestered by cytoplasmic calcium buffers. With entry and sequestration rates nearly the same, there would be little change in Ca^{2+} and little release of transmitter, just as Spencer *et al.* observe.

Although it seems that the basic mechanism of synaptic transmission may be universal the performance of each synapse, whether from goldfish, squid or jellyfish, depends on the shape of the presynaptic action potential, the voltage dependence and kinetics of the presynaptic calcium channel, and the kinetics of cellular regulation. More preparations are needed to evaluate the influence of these different factors. The *Polyorchis* preparation developed by Spencer and colleagues has the advantage of good electrical access to the synaptic sites and so further dissection of the presynaptic currents may be possible. But it is just one such preparation identified in jellyfish. Others are the neuromuscular synapse of *Aglantha*[11], with its dual system of action potentials, and the bidirectional synapse of *Cyanea*[12]. Researchers with patrons farsighted enough to support these ventures into the realms of the jellyfish have returned with great bounties. The advantages of using the simple nervous system of the jellyfish for experimentation are more than just travellers' tales.

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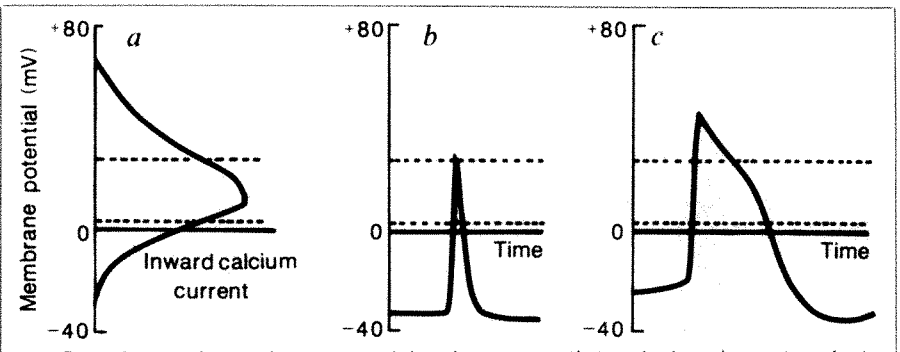

a, Step changes in membrane potential at the presynaptic terminal produce a transient inward calcium current across the terminal membrane. In this representation of data from *Polyorchis* motor neurons[3] the peak of the inward calcium current is shown on the horizontal axis with the membrane potential plotted vertically. The range of membrane potentials at which the calcium current is greater than 75 per cent of maximum lies between the dotted lines. In *Aplysia* neurons the relationship between inward calcium current and membrane potential is displaced to more positive voltages (upward in the figure); in squid axons the relationship is displaced downward. *b,c* Intracellularly recorded action potentials from *Polyorchis*[3] showing that the calcium influx is near maximum for almost the entire time that the smaller shorter action potential (*b*) overshoots 0 mV, whereas the longer action potential (*c*) enters this range only after a considerable delay.

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GALAXIES

Cosmic merger mania

E.S. Phinney

GALAXIES have sometimes been called island universes. But just as islands grow, crumble and move about on geological timescales, so galaxies grow, move and merge with one another on cosmological timescales. The *Dynamics and Interactions of Galaxies* was the avowed topic of a recent conference*, but the real theme was the question: to what extent are the properties of the galaxies we see today a result of the collisions and subsequent mergers of earlier generations of galaxies?

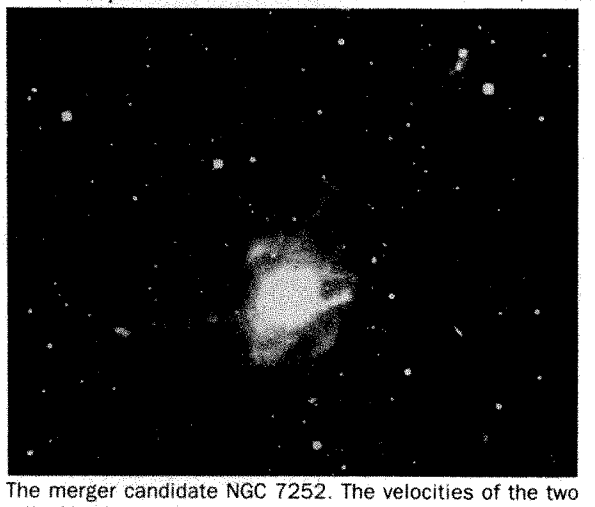
In the early 1970s, many people would have answered "entirely". A popular view was that small clusters of stars formed at high redshift z (relatively soon after the Big Bang, perhaps as early as $z \approx 10^3$), and repeatedly merged to form galaxies and clusters of galaxies. Unfortunately galaxies are clearly defined entities, not part of a self-similar hierarchy. So it later became popular to argue that galaxies sprang almost fully formed from the coherent collapse of high-density regions of the expanding Universe.

Throughout this dark age, a few — notably Alar Toomre[1] — maintained that mergers of galaxies must be important. Fewer than 1 per cent of the galaxies visible in our snapshot of the Universe are involved in the violent interactions that lead to coalescence, but those stages are shortlived. The actual number of galaxies that must have had such interactions is larger by the ratio of the age of the Universe to the lifetime of the evidence. Although no one has yet made a careful estimate using models of the interactions in a complete census of galaxies, the impression is that at least 10 per cent, and possibly a majority, of galaxies must have merged with other galaxies of similar size in the most recent half of the age of the Universe. Mergers were, if anything, more frequent in the past.

Small galaxies are much more numerous than big ones. Almost inevitably, every large spiral galaxy must have captured many smaller galaxies. Indeed, a steady dribble of gas is needed to maintain the open spiral arms so characteristic of gas-rich spiral galaxies (A. Toomre, Massachusetts Institute of Technology). The rings of gas orbiting the poles of some spiral galaxies are convincingly interpreted as the remains of the capture of a particularly loosely bound companion (L. Sparke, Univ. Wisconsin).

* Heidelberg Conference on Dynamics and Interactions of Galaxies, 29 May–2 June 1989, organized by SFB 328, University of Heidelberg.

The merger of two bright collections of stars can only lead to a still brighter collection (though in making comparisons, one must be careful to account for the subsequent evolution of younger stars present and perhaps created during the merger). The remnants of mergers should thus be found among known galaxies. Obvious candidates are elliptical galaxies and the piles of stars (bulges and spheroids) surrounding the disks of spiral galaxies. Improved instrumentation has uncovered subtle fossil evidence supporting this view. K. Freeman (Mount Stromlo Obs.) reviewed the curious properties of our own Milky Way. One of the most suggestive is the apparent absence of stars near the Sun with angular momentum per unit mass l larger than the Sun's, $l_\odot$. There are many stars with $l < -l_\odot$, but few with $l > l_\odot$. This anisotropy could be understood if the high-velocity stars were the disrupted constituents of small satellite galaxies captured by the Milky Way. Those satellites with orbits in the same sense as the rotation of our disk would be quickly pulled into the plane of the disk and their orbit circularized[2], so their disruption would leave no stars on significantly non-circular orbits. Satellites on retrograde orbits, by contrast, would interact only weakly with the disk, and their decaying orbits could remain eccentric. They would leave a population of retrograde high-velocity stars like that observed. B. Carney (Univ. North Carolina) pointed out that if such satellite captures were common in the early history of our Galaxy, the energy deposited could increase the scale height of the stars then in the disk so much that we would not now identify them as part of the disk. This might explain the embarrassing paucity of old low-metallicity stars in the disk (the famous 'G dwarf problem'); indeed the missing stars appear to be present in the so-called thick-disk population.



The merger candidate NGC 7252. The velocities of the two tails (the longer one is at least 4 Milky Way diameters in length) suggest that this galaxy is the result of a merger, begun about 10^9 years ago, of two disk galaxies[11]. Its spectrum shows younger stars than are found in a normal elliptical galaxy, probably created from gas compressed in the violent merger. The radial brightness profile and velocity dispersion are characteristic of an elliptical galaxy. In another 10^9 years, the young stars will have faded, the tails have dissipated and the loops have phase-mixed away. The result will probably be indistinguishable from an elliptical galaxy. (Photograph by F. Schweizer with the 4-m telescope at Cerro Tololo, Chile.)

Elliptical galaxies were once defined as featureless piles of stars. It now seems that most of the bright ones contain faint ripples, crosses and plumes of starlight (which correlate with spectroscopic evidence for young stars; F. Schweizer, Carnegie Inst.), all clearly evidence of recent capture of a disk galaxy (J.-L. Prieur, European Southern Obs.). Their faint disks and box-shaped isophotes (R. Bender, Landessternwarte Heidelberg), the peculiar twists and reversals of the streaming of their stars (S. Wagner, Landessternwarte Heidelberg), and their warped disks of gas (T. de Zeeuw, Calteen) may also be evidence of cannibalism.

Giant elliptical galaxies at the centres of galaxy clusters have long been suspected of growing by capture of cluster members. Estimates of the rate of capture in present clusters indicate, however, that no more than about a third of the stars in these central galaxies can be accounted for in this way (T. Lauer, Princeton Univ.; S. Tremaine, Canadian Inst. Theoretical Astrophysics). Fortunately, mergers of clusters of galaxies seem to be quite common. In such mergers, the heaviest galaxy of each cluster would tend to sink to the bottom of the combined potential well and merge with its competitor. Binary giant galaxies are seen in about a fifth of clusters and are expected to last for about a fifth the age of the Universe (Tremaine), so giant galaxies, like giant companies, may grow mainly by merging with other giants, not by capturing small fry.

About 1 per cent of galaxies lie in very compact groups with 2 or 3 others. The galaxies in these compact groups will collide and merge in a time difficult to estimate, but probably of the order of one-fifth of the age of the Universe (S. White, Steward Obs.). Numerical simulations of such mergers, now quite realistic[3], show that the result of merging two or more disk galaxies is an excellent facsimile of an elliptical galaxy (J. Barnes, Inst. Advanced Study; P. Quinn, Mount Stromlo Obs.).

The simulations have also mitigated the force of some long-standing objections to mergers[4]. The orbital energy of the merging galaxies is not shared equally by all the stars, nor does the merger mix stars

randomly: dynamical friction gives the orbital energy primarily to loosely bound stars and dark halo particles, ejecting some. Stars in the tightly bound cores of the pre-merger galaxies end up in the more tightly bound cores of the final system. Mergers thus preserve correlations of the properties of stars with their binding energies (M. Franx, Harvard Univ.).

Results from the Infrared Astronomical Satellite (IRAS) have recently drawn attention to the merger process itself. The easily simulated mergers of galaxies composed largely of stars are rather dull. Excitement is provided by gas, which can radiate, form stars and transport itself by forces stronger than that of gravity. The brightest galaxies in the infrared sky seem to be the products of recent violent interactions (R. Joseph, Imperial College; U. Klaas, Max Planck Inst.). Observations of molecular transitions at millimetre wavelengths with new interferometers show that the gas has been concentrated toward the centre of such galaxies[5], and heroic simulations have given us some understanding of how this occurs (M. Noguchi, Tokyo Univ.; F. Combes, Paris Obs.; L. Hernquist, Inst. Advanced Study). On a scale another 10^8-times smaller may lurk a central black hole. Ideas on how gas may lose enough angular momentum to reach it are still qualitative (E.S.P.; ref. 6), yet reach it the gas seems sometimes to do. Many, perhaps most, quasars and powerful radio galaxies show signs of recent interactions (A. Stockton, Inst. Astronomy, Hawaii; T. Heckman, Univ. Maryland). But few of the less dramatically active galaxies, like Seyferts, are obviously connected with interactions or mergers (J. van der Hulst, Groningen Univ.).

At the meeting, then, emerged a picture of the effects of collisions between present-day galaxies. Still lacking is a convincing calibration of the rates of collisions and the lifetimes of the various observed pre- and post-merger phases. Without this, we cannot say whether the present forms of galaxies were largely determined by the time the Universe was a tenth of its present age, or whether most galaxies have had their structure altered by more recent mergers. As G. Efstathiou (Oxford Univ.) emphasized in the opening lecture, the cold dark matter model, our only theory of galaxy formation still both viable and predictive, suggests the latter[7]. There are intriguing spectroscopic hints that many galaxies at a redshift of 0.5, when the Universe was about half its present age, were very different from galaxies today[8-10]. The much-delayed Space Telescope will tell us whether their shapes and sizes are different too. Yet proving that mergers are the cause, will require completing the tedious task of measuring the rates and lifetimes of galaxy–galaxy reactions. □

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PALAEONTOLOGY

A backbone for the vertebrates

Henry Gee

COMPARATIVE zoology is experiencing a quiet revolution. For the past 20 years, a view of chordate origins has been emerging which combines classical anatomy with modern cladistic theory. In the latest[1] of a long line of papers, R.P.S. Jefferies, with A.J. Craske, describes the systematic position of a fossil invertebrate called *Barrandeocarpus norvegicus* (Fig. 1) from the Upper Ordovician of Norway. Many researchers consider carpoids, the group to which *Barrandeocarpus* belongs, as a long-extinct group of echinoderms. But following earlier theoretical work[2], Jefferies and his co-workers have thrust the honour of vertebrate ancestry on this obscure group[3], calling them calcichordates[4] — that is, chordates with an endoskeleton of calcite, like echinoderms.

Arguably the most widely accepted view of chordate ancestry stems from Garstang[5,6], who believed that the distinctive 'tadpole' larva of tunicates was an innovation, interpolated between ciliated larva and sessile adult[7]. Vertebrates would have evolved from tadpole larvae by paedomorphosis[8]. Garstang's ideas were based on a meticulous organization of the data in what might almost be termed a cladistic way (see below). This rigour might explain why his ideas have stood the test of time.

Jefferies, on the other hand, believes that adult ancestral chordates were free-living, anatomically very much like tunicate tadpole larvae, but with a calcite endoskeleton. The sessile stage came later — a specialization of tunicates rather than a secondarily lost feature of chordates.

Although Jefferies can trace the order in which chordate features were acquired in a way that is consistent with chordate structure, he is hampered, like Garstang, by the lack of fossil evidence for the conceptual leap from the calcichordate to the recognizably vertebrate condition. The most advanced vertebrate ancestor known

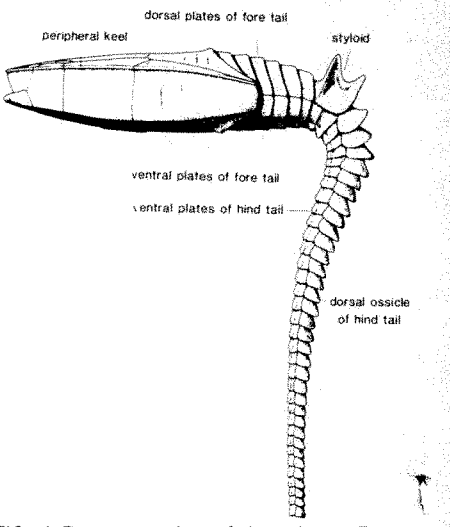

FIG. 1 Reconstruction of the mitrate *Barrandeocarpus norvegicus* in left lateral view, by A.J. Craske (from ref. 1). Jefferies (ref. 3) interprets the flexible, segmented tail region as being based around a notochord. The head region contains the brain and viscera, and the mouth is at the front (left of the figure).

among calcichordates is still a tunicate tadpole larva supported by calcite. No modern chordate has a calcite skeleton, and a radical revision of the body plan would necessarily follow its loss. This revision would be consistent with anatomical observation but rests on an extended chain of reasoning which at least one observer believes is stretched too far for credulity[9], despite the minute attention to detail at each stage.

What marks Jefferies' ideas out from competing hypotheses is the explicit use of cladistics — in particular, the way in which he applies cladistic methods to classifying fossil groups. Cladistics, an analytical method for reconstructing phylogenies, is based on identifying groups defined by sets of derived characters shared by all members. These groups are termed monophyletic[10]. But groups based on primitive characters, or the absence of derived characters, are termed paraphyletic because they cannot be defined uniquely on the basis of derived traits.

Coping with paraphyly is the thorniest problem in systematic palaeontology. It is relatively easy to avoid paraphyly when classifying extant organisms, but how can fossils be organized in a phylogenetically useful way? Some have sought to get round the problem by asserting that fossils are never informative enough to overturn

a phylogeny constructed using data from extant taxa[11–13]. In a News and Views article last year[14], I discussed how fossil species along a transformation series could be used to overturn neontologically based cladograms in certain circumstances[15]. But the transformation series is a paraphyletic concept derived from traditional, synthetic ideas which can be expressed in cladistic terms only by a careful choice of characters used to justify existing ideas of relationship[13]. Jefferies, on the other hand, meets paraphyly head on. Much of the discussion[1] about *Barrandeocarpus* concerns how this fossil form can be incorporated in a scheme embracing both paraphyly and transformation series.

Jefferies redefines a group of primitive carpoids called cornutes as the 'stem group' of chordates[3,16], through which runs the 'stem lineage' to more derived chordates in the 'crown group' (Fig. 2). Each evolutionary novelty along the stem lineage[16] forms the upper or lower bound of a discrete segment of the stem lineage, called a plesion. Stem groups and plesions are paraphyletic in that they cannot be defined by unique sets of derived characters that exclude crown groups. This is because the character that defines the lower bound of a stem group or plesion is also present, at least primitively, in more 'crownward' forms. Jefferies' use of the plesion concept moves it a considerable distance from its original definition (Fig. 2)[17]. His addition of concepts such as the stem lineage has turned the (monophyletic) plesion into a (paraphyletic) lineage segment, an idea he has worked out most extensively in the systematic placement of *Barrandeocarpus*[1].

Jefferies defines a relatively derived

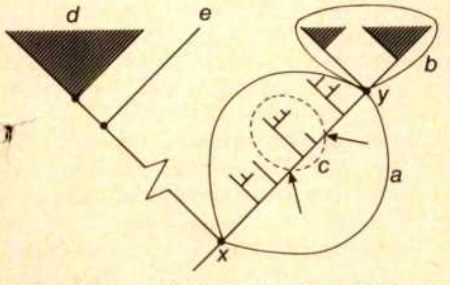

FIG. 2 Some cladistic terminology explained. The right hand side of the diagram shows a stem group *a*, (such as the cornutes), leading to a crown group *b* (such as the mitrates); *c* is a plesion (see ref. 1) defined by evolutionary novelties (heavy arrows) along the stem lineage (between *x* and *y*). The stem group and plesion are clearly paraphyletic. The left-hand side of the diagram illustrates the original plesion concept[17]. The fossil taxon *e* is incorporated in a character analysis built using data from living forms only to lie as a monophyletic sister group of *d*, which may contain extinct forms, living forms, or both; *e* is referred to as a plesion, but note that it is a monophyletic group used to express character distributions only, and not a paraphyletic group such as *c* which essentially summarizes a grade of organization.

Rock Festival at British Museum

THIS sample of aragonite from Cumberland features in a new exhibition at the Geological Museum in Cromwell Road, London SW7, now part of the British Museum (Natural History). The 'Rock Festival' features the world of minerals in nature and art and runs until 15 January 1990. Originally developed by the city of Strasbourg as part of its bimillenial celebrations, the exhibition also highlights many of the museum's own specimens. □

group of carpoids, called mitrates, as crown-group chordates which retain calcite skeletons. He suggests that there are three separate stem groups within the mitrates, leading, respectively, to acraniates (such as the amphioxus *Branchiostoma*), the tunicates and the vertebrates. Every known mitrate can be assigned to one of these stem groups[3]. *Barrandeocarpus* is the member of a plesion in the vertebrate stem group within mitrates, but is somewhat removed from the actual vertebrate stem lineage. Defining its precise position has led to a new method of subdividing plesions[1].

Jefferies' theory is by no means generally accepted[9], and the loss of the calcite skeleton is a particular stumbling block. Jefferies claims that calcite resorption can be seen in some mitrates such as *Mitrocystella*, but even if calcite had been lost in this way, many questions about the nature of the calcichordate integument remain unanswered. The only living animals with a calcichordate-type skeleton are echinoderms, and their integuments are fundamentally different in structure and function from those of chordates. Furthermore, the calcichordate fossil record seems to be at variance with the phylogeny reconstructed by Jefferies[3]. Calcichordates are known from Lower Palaeozoic rocks throughout the world, but almost all known examples postdate the earliest records of true, fish-like vertebrates. So if the calcichordate theory is correct, many examples remain to be discovered in Cambrian and Precambrian rocks. (Indeed, new species of calcichordate are still being found in Wales in the United Kingdom, where Lower Palaeozoic rocks are well-charted[18].) Problems of completeness in the fossil record are best solved by digging rather than theorizing — but making the calcichordate theory fit the harsh realities of the fossil record may turn out to be its biggest difficulty.

In the meantime, the fossil species Jefferies uses to build his theory[3] can be considered as abstract and timeless types rather than as real species, between which there are not evolutionary relationships but only affinities. Pre-evolutionary biologists (such as Linnaeus) classified organisms fairly well without invoking evolutionary change. Cladists accept that evolution happened, but prefer to ignore it as an explanatory process until its pattern has been worked out.

Nevertheless, others (including some of Jefferies' critics) think that the calcichordate theory — as a theory — is the best so far to explain chordate and vertebrate origins, in that it is the most self-consistent. For all its problems, and whether one believes it or not, the organization of an otherwise bewildering array of meticulously well-defined characters according to cladistic principles makes the calcichordate theory a hard act to follow. □

Henry Gee is on the editorial staff of Nature

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NUCLEAR PHYSICS

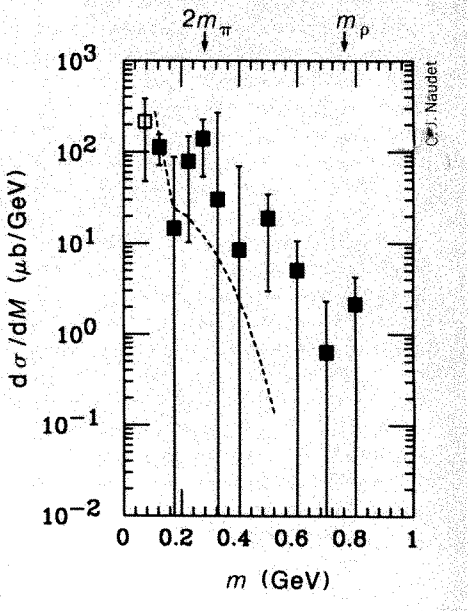
Heavy photons light up hot nuclei

Philip J. Siemens

By colliding atomic nuclei at relativistic velocities, physicists heat and compress matter to temperatures and densities not seen since the first millisecond of the Big Bang. In the 10 August issue of *Physics Letters* B, Roche *et al.*[1] report new observations of rare electromagnetic signals that promise to illuminate the properties of hot, dense matter made in nuclear collisions. The electron–positron ($e^+ - e^-$) pairs they measure carry information about the electric currents in the matter. These data are especially valuable because the pairs are among the few signals able to penetrate the hot, dense matter. The pairs are the material remnants of massive photons, quanta of radiation that exist only ephemerally in the neighbourhood of the currents that produce their electromagnetic fields. The experiments seem to confirm theoretical predictions by Gale and Kapusta[2] and others[3] that the currents from charged mesons, principal agents of the nuclear force, would be seen as bumps in the spectrum of photon masses. Refinements of these measurements could provide unique insight into how mesons behave in the hot, dense matter, which is the key issue unresolved by previous observations of nuclear collisions.

The ability of the $e^+ - e^-$ pairs to penetrate the hot, dense matter that creates them offers hope of a breakthrough in the huge programme of experiments in which hot, dense matter has been made and studied in the Lawrence Berkeley Laboratory since 1975. A complex of linked accelerators provides beams of nuclei moving as fast as 95 per cent of the speed of light. When these nuclei collide with nuclei in stationary targets, the force of the collision compresses them to a fraction of their original volume, producing matter densities of about 10^{15} g cm^{-3}. In the collision, much of the energy initially imparted by the accelerator is converted to heat, leading to temperatures of about 10^{12} K (for comparison, the core of a type II supernova reaches comparable densities but at much lower temperatures). Unfortunately, this hot, dense matter explodes, so that within 10^{-22} seconds its density has dropped below that of the original nuclei from which it was formed, though its temperature is still around 5×10^{11} K. Until now, observations have been limited to the dilute, cool products of the explosion. Although the properties of the hot, dense matter determine the results of the explosion, the necessary theoretical analysis is so indirect that large uncertainties remain in the deduced quantities: compression energy, heat capacity, collision and reaction rates, and the potential energies of particles in the matter all remain uncertain within a factor of two.

The electrons and positrons observed by Roche *et al.*[1], like their parent photons, remain undisturbed by the violent rearrangement of the matter during the explosion, because they are unaffected by the strong nuclear forces. This advantage is countered by an experimental disadvantage: because the forces that create the pairs are so small, the average time needed to create a pair greatly exceeds the duration of the explosion, and hundreds of explosions must be monitored before an interesting pair is created. The resulting low rates place rigorous demands on experimental technique. Nevertheless, an impressive effort has now led to results for nuclear collisions that seem to contain new information about the properties of the hot, dense matter. These results follow close on the heels of a report[4] in *Physical Review Letters* demonstrating the method for collisions of protons on beryllium targets. The importance of the new technique is highlighted by theoretical arguments that certain characteristics of the pairs should be especially sensitive to some of the most interesting and controversial properties of the hot, dense matter.



The invariant-mass spectrum for the reaction of a beam of Ca nuclei at 0.95*c* on a Ca target. Solid squares: experimental measurements; open square: tenative analysis; dashed line: contribution due to single-pion decay.

Because momentum, p, and energy, E, are conserved, the p and E of the $e^+ - e^-$ pair must come from the currents that created them. The pairs may be classified by their invariant mass $m = \sqrt{E^2/c^4 - p^2/c^2}$ which coincides with the mass of the photon that mediates their creation (see box). Pairs with small m are quite numerous, arising from 'bremsstrahlung' radiation due to the acceleration of colliding protons and from an infrequent decay mode of neutral π mesons (pions); the number of pairs from these sources decreases rapidly with m. Pairs with large m are very rare, originating in the annihilation of quark–antiquark pairs. The decay of the ρ meson produces pairs with that meson's mass, $m_\rho = 770$ MeV c^{-2}.

In addition to these mundane sources of $e^+ - e^-$ pairs, Roche *et al.* observe (see figure) many pairs with m less than m_ρ but greater than twice the pion mass m_π. Such pairs can be produced by the annihilation of charged pions $\pi^+\pi^- \rightarrow e^+e^-$. Pairs from pion annihilation would be especially interesting for two reasons: the pion is the main agent of the attractive force that binds nuclei; and the creation of pions is thought to be responsible for much of the heat capacity of nuclear matter. Not surprisingly, pions are strongly influenced by the presence of nuclear matter. The nature and extent of this influence is the most difficult point left unsettled by earlier experiments on colliding nuclei. Because the pions are so crucial to the properties of the matter, the controversy about their behaviour results in uncertainties in the deduced values of all the properties of hot, dense matter.

In 1987 Gale and Kapusta[2] pointed out

Heavy light **Because $e^+ - e^-$ pairs experience only the electromagnetic interaction, they have to be produced by way of photons, the quanta of the electromagnetic field. An ordinary photon, which propagates at the speed of light c, has no mass; a massive photon, possible only because of the uncertainty principle, can neither exist for long nor propagate far. Every photon, massive or light, is created by an electromagnetic current which provides the photon's momentum and energy. Because momentum p and energy E are conserved, each pair has the same p and E as the photon which produced it; the photon, in turn, receives that momentum and energy from the sources of the current. According to quantum mechanics, the momentum of a particle produced in a localized region of size Δx is indeterminate by an amount $\Delta p = \hbar/\Delta x$; similarly, the energy of a particle existing for a time Δt is indeterminate by $\Delta E = \hbar/\Delta t$ ($\hbar$ is Planck's constant). Thus an electromagnetic field existing in a limited region for a limited time has to be described by quanta — photons — whose energies and momenta are not related by the usual $E = pc$. Although such a photon, with a non-zero mass $m = \sqrt{E^2/c^4 - p^2/c^2}$, exists only ephemerally, that brief appearance gives it the possibility of creating an $e^+ - e^-$ pair for which both the electron and positron fulfil the required relations between momentum and energy so that they can continue to exist indefinitely.** P.J.S.

that the influence of nuclear matter on pions should be seen directly in the spectrum of masses of $e^+ - e^-$ pairs from their annihilation. In the absense of matter, the minimum mass of the pair would be $2m_\pi$ = 279 MeV. To a first approximation, one might expect hardly any pairs to be created with this minimum mass, both because the masses of most pairs would be increased by the kinetic energy of the pions and because the electromagnetic current, which is proportional to the velocity of the pions, vanishes when they have no kinetic energy. Thus the spectrum of masses would show[5] a peak well above $2m_\pi$ followed by another at m_ρ.

However, the energy of pions in nuclear matter is reduced by an attractive potential energy so that, as Gale and Kapusta argued, the position and shape of the two-pion peak in the $e^+ - e^-$ mass spectrum could show the magnitude of the interaction energy. The attraction increases so rapidly with the pions' momentum that the kinetic energy of a pion in the middle of a large nucleus is approximately cancelled by the potential energy[6] except for those whose momentum p is several times $m_\pi c$. As a result, pions with a wide range of momenta have about the same energy $m_\pi c^2$ in nuclear matter; any two such pions could annihilate to give an $e^+ - e^-$ pair with a mass near $2m_\pi$ (or even less because of the pair's momentum). In dense matter, the pions' attraction could be greater, leading to many pairs of even lower mass.

The figure shows that the spectrum of $e^+ - e^-$ masses measured by Roche *et al.* indeed exhibits a peak near $2m_\pi$, although the statistical uncertainties of the small data sample preclude a precise determination of its location. In the reaction of protons on Be at 4.9 and 2.1 GeV, similar peaks were observed[4] with better accuracy; after accounting for the low-mass pairs from decay of single pions, those peaks appear to be very close to $2m_\pi$ as expected for nuclear matter at normal density. The interpretation that these pairs arise from two-pion annihilation is confirmed by the observation[4] that a 1-GeV proton on Be produces only pairs consistent with the single-pion background. This is as expected because 1-GeV protons seldom produce two pions.

Encouraged by their success, Roche *et al.* are pressing ahead with their experimental programme. Before conducting further measurements on nuclear collisions, they are preparing an experiment to study carefully the production of $e^+ - e^-$ pairs in collisions of protons with hydrogen targets to enable an accurate evaluation of how many low-mass pairs are due to bremsstrahlung. Because the bremsstrahlung rate must fall smoothly with mass, it cannot be responsible for the observed peak in the mass spectrum; however, a detailed analysis of the shape and position of the pion-annihilation peak requires subtracting the more pedestrian processes (the single-pion decay is well in hand). After these studies, Roche *et al.* hope to return to the application of their technique to nuclear collisions, both in Berkeley and at the higher-energy beams now becoming available in Brookhaven. Theorists are also scurrying to extend their models to interpret these new measurements, which may contain the clue to the riddle of hot, dense matter. □

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CONSERVATION

Desert rhinos dehorned

N. Leader-Williams

THE dehorning of rhinos has finally been attempted in a brave experiment aimed at thwarting poachers[1]. Africa's black rhinos *Diceros bicornis* have dwindled at an unprecedented rate, from an estimated 65,000 in 1970 to fewer than 4,000 now, chiefly because of international demand for rhino horn. During the past decade conservationists' attempts to halt this decline using both a total trade ban on horn, enforced through the Convention on Trade in Endangered Species, and underfunded field-based projects to protect large populations *in situ*, have failed miserably[2,3]. The dehorning experiment was conducted in Damaraland, Namibia, where a small population of black rhinos uniquely adapted to desert life had once again become threatened when 16 of them were killed this year[1,4].

The experiment will cause controversy among conservationists because, although dehorning has been discussed as a measure to prevent poaching since the 1950s, until now it has been discarded in most areas of Africa for several reasons[5]. First, the cost of dehorning several thousand rhinos over tens of thousands of square kilometers would be extremely expensive. Second, the two African species, the black and the white (*Ceratotherium simum*) rhino, use their horns in sparring[6] and to defend calves against predators such as lions and spotted hyenas (see figure). Hence, hornless rhinos may be unable to maintain their social status or to rear their calves successfully. As important, most black rhinos live in thick bush, and a poacher sighting only a part silhouette could shoot before finding his quarry is hornless[5].

To stand the maximum chance of success, therefore, dehorning should be carried out in a small and discrete population of rhinos living in an open area where there are no natural predators. These conditions are met by the habitat and rhino population of Damaraland, where the principal factors to be considered are the effect of dehorning on social status and the risks of injury in fights with horned rivals. The other major advantage of Damaraland is that the successful conservation measures that brought the population of desert rhinos up from a heavily exploited population of around 40 in 1980/81 to 100 in 1988 involved local tribesmen who act as auxillary game guards[4]. Their role in informing potential poachers that rhinos in the area are now dehorned will be vital.

If successful in Damaraland, dehorning may become more widespread. Plans have already been made to dehorn a group of threatened white rhinos in Zimbabwe[7]. Even though previously rejected as a conservation method for the once large populations of black rhinos living in wooded areas, circumstances may have changed sufficiently for dehorning to be reconsidered in East and Central Africa. Remnant black rhinos in Kenya have been moved during the past few years into small fenced sanctuaries, a strategy which has so far been successful in stabilizing numbers[5]. As a further precau-

A mother rhino defends her bloodied calf against a hyena. Will dehorning increase predation risk? (Photograph courtesy of Dr Hans Kruuk, Banchory Research Station, Kincardineshire, UK.)

tion, however, all the enclosed rhinos could be dehorned, predators of rhino calves removed from the sanctuaries, and information and education campaigns mounted to inform poachers of the dehorning so that reprisal killings[8] are avoided. Tourists, too, would need to accept that hornless rhinos are better than no rhinos.

One other problem remains to be solved. The horns of the Damaraland rhinos have only been sawn off and filed down[1], and so will regrow within two to three years. If initial dehorning experiments prove successful and rhinos are not to be exposed to the risks of repeated immobilization, cauterization of the horn bases should be experimented with. An analagous operation, albeit with horns of a somewhat different structure, is carried out routinely to poll horned breeds of cows and is permanent[9]. Conservationists can only hope that Namibia's courageous move will play a constructive role in a final stand to save Africa's rhinos over the next decade. □

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GEOLOGY

Fluid flow through fault zones

Andrew M. McCaig

NEW work in Virginia[1] emphasizes the importance of fault zones as major conduits for fluid flow through the middle crust, and may provide a useful new tool for gold exploration. Gates and Gundersen[1] have found significantly enhanced concentrations of radon gas in soils overlying the Brookneal fault zone in West Virginia, relating this to uranium concentrations of 7–8 parts per million (p.p.m.) contrasted with the 1–2 p.p.m. found in nearby undeformed granite. There is also a rough correlation between the shear strain in the zone and the uranium and thorium content, suggesting a progressive increase in uranium enrichment with deformation.

It has been known for many years that mylonitic rocks in ductile shear zones are frequently very different in composition to the rocks they cut[2–5]. These chemical changes are generally ascribed to the passage of large volumes of hydrous fluid through the zones. Shear zones are by definition much more strongly deformed than surrounding rocks and are typically produced when volumes of rock metamorphosed or intruded at high temperatures are reworked under lower-temperature conditions. There is almost certainly a complex feedback mechanism between fluid flow, chemical alteration and strain concentration[5], but it is very hard to separate cause from effect. Nevertheless, it is clear that the only permeable pathways through large volumes of the continental crust must be shear zones, and that in many cases these will be effective fluid conduits only during active deformation.

It is interesting to speculate on the source of fluid and the driving force for fluid movement in shear zones. In the Appalachians, large blocks of highly metamorphosed basement rock such as the Blue Ridge and Piedmont belts (see figure) have been thrust for tens or even hundreds of kilometres over relatively unmetamorphosed sediments[6]. It is possible that the fluid moving through the Brookneal and similar zones was squeezed out from such sediments owing to the enormous weight of thrust sheets above them[4]. But the Brookneal Zone shows mainly strike-slip motion and was probably active before the main Alleghenian collisional event[7], which placed the Piedmont Belt over continental margin sediments. In these circumstances, fluid may have entered the shear zone as a result of seismic pumping[8], either directly or via an underlying decollement[9]. Seismic pumping occurs because of the development of microcracks in highly stressed rocks immediately before earthquake rupture. This increase in pore volume (dilatancy) leads to pressure differentials and sucking of fluid into the stressed rock. When rupture occurs, the microcracks close up, forcing out fluid and leading to greatly increased spring discharges[8].

Fluid movement through fault and shear zones is an important subject of study for both environmental and economic reasons. As pointed out by Gates and Gundersen[1], radon gas is a major and insidious environmental health problem in any area where uranium is concentrated in underlying rocks. Clearly, shear zones should be included in the list of potentially hazardous sites. Equally important is the question of waste disposal; there is hardly any area of crust which does not contain either ancient or currently active fault zones. It is vital to assess the role of seismic pumping if waste is to be buried in seismically active areas such as California, and the long-term permeability of fault and shear zones must be established even in stable regions. A matter of current concern in waste disposal is the presence of corrosive hypersaline brines as groundwaters in crystalline rocks[10]. One possible source for such groundwaters is leaching of ancient fluid inclusions[11]. Recent work in Leeds (S. Tempest, personal communication) shows that fluid inclusions from altered shear zones can contain up to 40 per cent by weight equivalent of NaCl. The possibility that shear zones may contain anomalous concentrations of ancient, corrosive fluids has implications for many engineering projects.

Finally, fault and shear zones are prime targets for gold exploration in many parts of the world. Mineralization is commonly associated with specific geometrical features such as bends and intersections of zones[12]. Often the location and geometry of shear zones are concealed by superficial deposits so that soil sampling for radon may prove a powerful exploration tool. □

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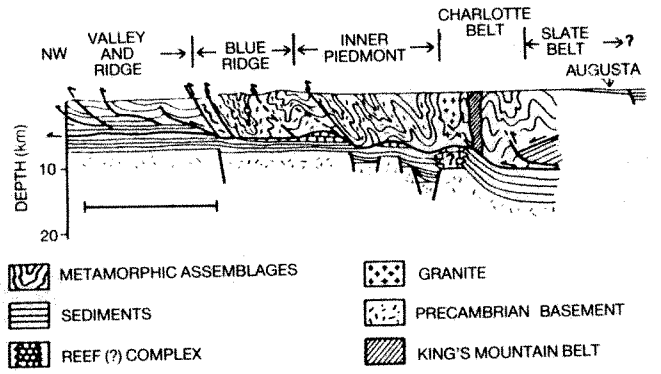

Cross-section through the southern Appalachians based on a COCORP seismic reflection profile. The Piedmont belt, containing the Brookneal shear zone studied by Gates and Gundersen[1], is interpreted to overlie unmetamorphosed continental margin sediments. (From ref. 6.).

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MEMBRANE PROTEINS

Detergent ringing true as a model for membranes

J. Barber

It is a frustrating business trying to understand how specific lipid species seem to optimize the functional properties of proteins that are embedded in the lipid bilayers that constitute most biological membranes. The problem is that membrane proteins are hydrophobic and therefore difficult to isolate and purify without using detergents to disrupt the membrane. Consequently, detergent molecules replace the lipid, and the best one can hope for is that information — for example, from high resolution X-ray crystallography — on how the detergent is organized in relationship to the protein will be of some relevance to how the natural lipids are organized. Even then, the approach is hampered by the fact that detergent molecules will often not form ordered arrays because of thermal motion, particularly in their hydrocarbon tails. On page 659 of this issue[1], Roth *et al.* report an alternative approach, which makes use of neutron scattering.

Roth *et al.* have studied highly-ordered crystals of the reaction centre of the photosynthetic bacterium *Rhodopseudomonas viridis*, isolated with the detergent lauryl dimethyl amine oxide (LDAO). X-ray defraction of these crystals has shown that the reaction centre has a hydrophobic core consisting of eleven membrane-spanning α-helices, five each from the L- and M-subunits of the protein and one from the H-subunit[2]. To be able to investigate the arrangement of detergent in the crystals, Roth *et al.* use the trick of varying the H_2O/D_2O content of the crystals, recording variations of the phase of the structure factor as a fraction of contrast.

Unlike X-ray crystallography, this approach gives the low-resolution data needed to identify the domains formed by the detergent molecules in the crystals. Under optimal conditions, the authors observe the detergent as micellular rings around the transmembrane α-helices of all three subunits. The thickness of the detergent rings along the α-helices is 25–30 Å, about twice the length of an LDAO molecule. Therefore, it seems that LDAO arranges itself around the hydrophobic core of the protein complex in the way depicted in text books for natural membrane lipids — as a bilayer.

Recently, Yeates *et al.*[3] have conducted minimum energy calculations for the possible organization within a lipid bilayer of the reaction centre of *R. sphaeroides*, which has an atomic-resolution structure[4] that is remarkably similar to that of the *R. viridis* reaction centre. Their analysis, however, predicts a thicker hydrophobic region for lipid-protein interactions of about 40–45 Å. The most likely explanation for this difference is that the bacterial reaction centre does not exist as a separate entity within the membrane but normally forms part of a much larger complex involving the proteins that bind the light-harvesting bacteriochlorophylls[5].

The neutron-scattering experiments of Roth *et al.* have also shown how the detergent domains interconnect throughout the crystal lattice. The results quite clearly show that LDAO not only interacts stereo-specifically with the hydrophobic region of the reaction centre but also creates optimal spacing for direct interactions to occur between the hydrophilic regions of adjacent proteins. Apparently, it is the interplay of these two factors that is vital for obtaining highly ordered lattices.

The message, therefore, is that there is no 'magic' detergent for crystallizing membrane proteins and each system must be explored independently. Moreover, it is likely that a similar message will apply to the packaging and organization of membrane proteins in their natural environment and that this underlies why nature has not been able to evolve a 'standard' lipid species for all systems. □

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FLUID MECHANICS

Oil slicks still the waves

John C. Scott

The effect of oil in calming waves is not only one of the oldest phenomena in the scientific calendar[1], it is one which still excites much interest. A recent paper by Alpers and Huhnerfuss[2] provides a timely summary of recent progress in understanding the calming effect of oil films on the sea surface. It also reports important progress in establishing the link between the processes of wind-energy input, wave-energy re-distribution and energy removal.

Contrary to popular belief not all slicks come from spilt crude oil. The ocean's natural productivity produces much harmless wave-damping material, and it is vital to distinguish the two causes. Concern over pollution, interest in the climatic influence of the sea surface, and the advent of powerful methods of observing the sea from space all ensure continuation of our interest in wave-damping effects.

The basic mechanism of wave damping was given by Horace Lamb, in the 1895 edition of his *Hydrodynamics*[3], although the finer points still attract attention (and some controversy). Surface-active chemicals reduce the surface tension of water and the effect increases with the surface concentration of the chemical; when a wave stretches and compresses the surface it increases and decreases the surface tension, causing tangential boundary stresses which always oppose the wave motion. The film gives the surface a 'dilational elasticity'; in the language of linear hydrodynamics the surface boundary condition changes from a 'free' surface to one which supports tangential stress, and a significant viscous boundary layer results. Note that the lost wave energy passes (as heat) into the water: it does not directly heat the film.

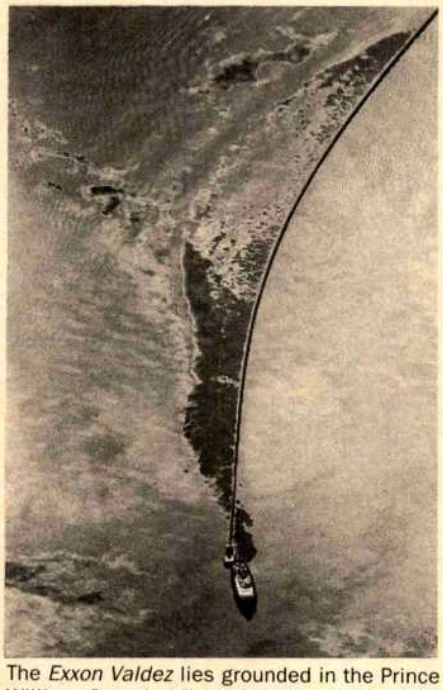
Inextensibility is the upper limit of elasticity, although real films never reach this. One of the most interesting effects of films is that the maximum damping can occur at quite small values of elasticity. Perhaps most remarkable is that the most effective films come from insoluble materials spread as single molecular layers; the effect seems quite disproportionate in this light.

A film usually gives a sharp damping peak, about twice that predicted for the inextensible case, and peaks appear in the variation of damping with wave frequency as well as with film elasticity. Alpers and Huhnerfuss prefer to consider these peaks as 'resonant' energy exchange between two wave modes — one the normal transverse surface waves and the other a highly damped longitudinal wave where motion is mainly to and fro at the horizontal surface. These latter waves are often called Marangoni waves, honouring the contribution to surface-film studies made by the nineteenth century Italian physicist. Although it is often found intuitively useful for considering the damping peaks, this division of the hydrodynamic solutions is not necessary (N. Thomas, personal communication). The conclusions reached by Alpers and Huhnerfuss are, however, not affected by this.

One further interesting influence on wave damping is that the film need not be purely elastic. Viscoelasticity can arise from molecular factors in the film, and

from film solubility, which allows time-dependent diffusive interchange between surface and bulk, and acts to reduce the surface tension gradients.

These considerations relate to all waves propagating on water. However, the wind-blown ocean is clearly more complicated. The primary damping effects of surface films are usually found with wavelengths less than about 100 mm, whereas wind-tunnel and sea experiments on wind



The *Exxon Valdez* lies grounded in the Prince William Sound, off Alaska. The thick slicks formed by crude oil are less surface active than biogenic slicks, and may be distinguished by analytic techniques.

waves all show very large effects on waves several metres long[2].

The novel step taken by Alpers and Huhnerfuss is to bring together wave-damping theory with recent results on energy exchange within the surface-wave spectrum, in particular nonlinear wave–wave interactions. The authors draw on recent results by Hasselmann and Hasselmann[4] and Phillips[5] concerning the distribution of energy within the spectrum and the rate of energy input from the wind.

The mechanism proposed follows the existence of the damping peak in the frequency variation, often near 5 Hz. Wave spectra on film-covered surfaces are duly found to have a pronounced dip at the corresponding wavelength (50–100 mm). It is proposed that the effect at much longer wavelengths comes from nonlinear wave interactions acting to re-establish the normal equilibrium spectrum, draining energy into the region of the minimum, and away from the longer waves. The authors present approximate calculations which suggest that such a mechanism would indeed explain the observations. They also give preliminary computations in support of the conclusion, although truly quantitative results were not possible because of computer limitations.

The linking of two such complex theoretical areas as film damping and wave–wave interactions is not straightforward, and several marginal assumptions have to be made, such as that the nonlinear process itself is not primarily affected by the film, and that other energy 'sinks' such as wave breaking are unaffected. Nevertheless, the results are encouraging when the calculations are compared with laboratory and sea data. The latter include both wave-amplitude and radar backscatter measurements, these being crucially important for the detectability of slicks by real-aperture or synthetic-aperture radars.

Experience of some form of slicks is common. Sun-tan oil can be seen to spread from summer bathers, and on very hot days intricate near-shore patterns of slicks can be seen. These are almost certainly from natural underwater biological activity — fish or plant life — rather than pollution. Even many hundreds of miles from civilization, a calm sunny day will give a 'glassy' sea. Such slicks are frequently unfairly attributed to polluters, and it is therefore vitally important to discriminate slick causes.

Fortunately, major pollutants such as crude oil (see figure), fuel oils and lubricants are usually quite different from biogenic slick materials. They are much less surface active, and they therefore tend to form thicker layers, spreading less. This leads to differences in radar backscatter, and Alpers and Huhnerfuss cite a paper in press on just this topic. There can be even more significant differences in the lidar, thermal infrared and fluorescent ultraviolet signatures of slicks, which could be detected by a multisensor approach, such as that adopted in the United Kingdom by the Warren Spring Laboratory.

Slicks also play an important role in ocean research. Natural slicks have a tendency to become aligned with regions of strong shear, and a remote sensing technique such as imaging radar[6] or sunglint[7] can thus provide valuable oceanographic information. The increasing availability of space-borne sensors, such as the radar-imaging satellite ERS–1, will ensure interest in surface films for many years to come. □

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DAEDALUS

Flying dust

MODERN detergents have done much to ease the never-ending problem of keeping things clean. A detergent molecule has an oleophilic end which binds to a greasy dirt particle, and a hydrophilic end which remains solvated by the wash water. So the adhering molecules 'couple' the dirt particles to the water. They lift off the surface to be cleaned, and come into liquid suspension.

Daedalus now points out that exactly the same mechanism could apply in air. Dust is hard to blow off a surface because its particles are poorly coupled to the air. If they could be covered by adsorbed molecules which interacted strongly with the air, they would lift off much more readily. So Daedalus is inventing a gaseous detergent.

He is synthesizing molecules whose hydrocarbon 'head' readily condenses onto greasy dirt. Their 'tail' is some long, light, flexible chain presenting a big collision cross-section to air molecules, probably a fluorocarbon (hydrocarbon chains, sadly, are oleophilic). Many fluorocarbons are extremely volatile for their molecular weight, and should have a powerful air-lift action on dirt particles.

But the new detergents will not be a gas. For by analogy with water-based detergents, it should work best at concentrations around 0.1 per cent, or a partial pressure of 0.001 atmospheres. And since it must condense out of the air and be adsorbed hygroscopically on the dirt at this concentration, this must be its saturated-vapour pressure at room temperature: implying a boiling-point around 180 °C. So 'Aerosolve' (as it will be called) will have quite a high molecular weight, with room for much chemical subtlety.

Its first mass application will be as a vacuum-cleaning aid, wafted ahead of the machine by an evaporator on its cleaning head. The stickiest dust will lost its grip on the carpet and be effortlessly sucked up. More radically, a room permanently saturated with Aerosolve would never get dirty in the first place, for the dust would stay harmlessly in air-suspension. Just hang a wick or paper strip saturated with Aerosolve in the room, and your cleaning problems are solved. The gradual insidious dirtying of all surfaces, which only becomes apparent when you rub a finger over them, or take a picture down and find a clean patch behind it, would simply not happen. The slow fouling of air-cooled laboratory equipment, which makes the servicing of old computers, and so on, such a grimy experience, could similarly be prevented.

Even better, Aerosolve vapour in the air should prevent dust and bacteria being deposited in the lungs as well. The benevolent detergent-vapour could thus help to prevent pneumonia, asbestosis, and some of the nasty diseases that afflict smokers.

David Jones

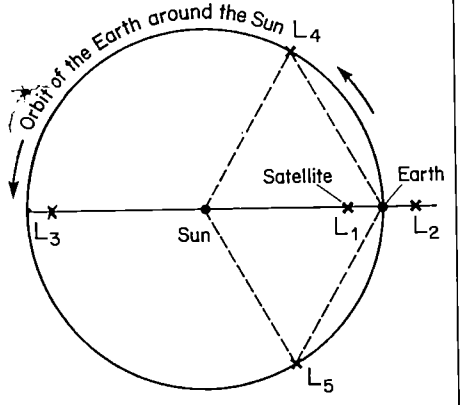
Mirrors to halt global warming?

SIR—It seems very likely that little will be done in the future to reduce the emission of greenhouse gases. If global warming is confirmed, a remote but feasible possibility to reverse the temperature increase is by changing artificially the solar radiation incident on the Earth.

The radiation balance of the Earth is controlled by the Stefan–Boltzmann law:

$$\varepsilon\sigma T^4 = (1-A)S_0/4 \qquad (1)$$

where the left-hand side is the infrared black-body radiation from the Earth and the right-hand side represents the mean solar (visible) radiation on the Earth. Here A (=0.3) is the albedo of the Earth, S_0 (= 1.372 kW m^{-2}) is the solar constant near the Earth, σ (= 5.67×10^{-8} W m^{-2} K^4) is the Stefan–Boltzmann constant, and ε (= 0.62) is the emissivity of the Earth with its atmosphere. Equation (1) gives T = 288 K as the mean temperature on the Earth's surface.



The five Lagrange points L_1–L_5 for the three-body system (Sun, Earth and satellite). In our case L_1 is chosen as the position for the mirror-bearing satellite.

To compensate for a temperature increase of ΔT originating from the greenhouse effect, we have to reduce the relative radiation ΔS_0 by

$$\frac{\Delta S_0}{S_0} = 4\frac{\Delta T}{T} \qquad (2)$$

For example, to compensate for a temperature increase of 2.5 K, the solar radiation must be reduced by about 3.5%. The task could be done by satellites bearing large lightweight mirrors. However, satellites in 'normal' orbits do not cast their shadows permanently on the Earth.

This difficulty may be overcome, and the 3.5% reduction achieved, with a minimum mirror area of 4.5×10^6 km^2, by positioning a satellite in such a way that it will always stand between the Sun and the Earth, permanently casting its shadow on the Earth. In this context my attention has been drawn to some particular solutions of the three-body problem, the so-called Lagrange points.

In the plane of the elliptical orbit of a planet around the Sun there are five Lagrange points, L_1 to L_5 (see figure), which are characterized by the fact that if a third body (a satellite, for example) is positioned at one of these points, the geometrical figure between the body, the Sun and the Earth remains constantly self-similar. For our purpose the so-called collinear solutions, based on L_1, L_2 and L_3, are the most important, particularly Lagrange point L_1. If a satellite was positioned at L_1 it would remain on a straight line between the Sun and the Earth at all times and could cast its shadow permanently on the Earth. Thus, this would make possible a reduction of the solar radiation power on the Earth using a minimum of mirrors.

If we assume that the mass of the satellite is negligible relative to the mass of the Earth, then the distance between L_1 and the Earth is $d \approx 1.5 \times 10^6$ km, or about four times the distance between the Moon and the Earth (K. Stumpff *Himmelsmechanik* Vol. II, 104; VEB, Berlin, 1965). The satellite would have an infinite synodical period and its sidereal period would be the same as the Earth's (that is, one year).

How much energy will be required to bring mirrors of the necessary size to the point L_1? If we assume that they consist of aluminium of mass 10 g m^{-2}, at least 45 million tonnes of material will have to be brought to L_1. We estimate that the energy required to do this is equivalent to the output of 30 nuclear power stations producing 1 GW for 20 years. A very crude estimate of the cost of this venture gives, over 20 years, a figure of 6% of the present annual gross national product of the world per year, which is roughly equivalent to its total annual military expenditure.

It should be noted that the Lagrange position L_1 is an unstable position: there is no negative feedback mechanism to hold the satellite in position should it drift away for any reason, for example because of the light pressure of the Sun on the mirror. Furthermore, the Lagrange solutions are strictly valid only for a pure three-body problem. Although the attractive force of the moon on L_1 is at most only 2% of that of the Earth, this will have some influence. Thus the satellite would have to be stabilized actively, which requires permanent observation and maintenance. However, the solar light pressure is just strong enough to allow a robust active control of the equilibrium of the mirror.

Of course, there are other important aspects which have not been considered, the most critical of which is whether it is possible to produce reflecting foils with a mass of only 10 g m^{-2} which will be able to withstand mechanical forces, solar winds and so on for a long time.

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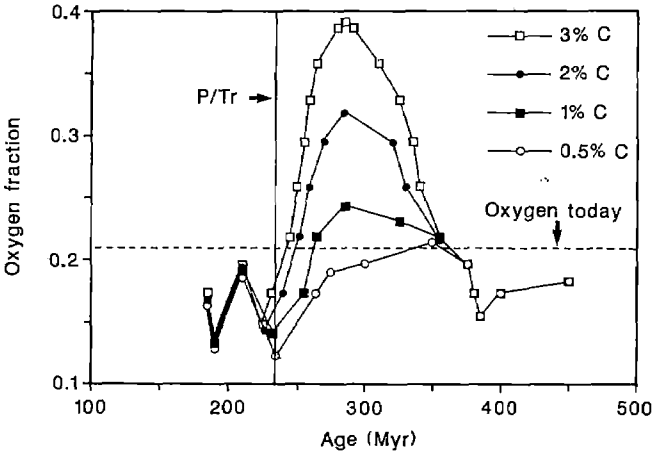
Drying, O_2 and mass extinction

SIR—Reports[1,2] of a drop in the $^{13}C/^{12}C$ ratio of the oceans during the late Permian have been interpreted as a result of an input of light carbon to the oceans from the oxidative weathering of pre-existing organic matter, either on the ocean floor[2] or on the continents[1]. Increased weathering on the continents is attributed to the lowering of sea level and the exposure of additional organic-containing sediments to the atmosphere at that time[1]. Weathering of organic matter results in the uptake of O_2 from the atmosphere, and a lowering of atmospheric oxygen might have contributed to the Permo/Triassic extinction[2], the most extensive mass extinction to occur over all of Phanaerozoic time[3].

Although increased organic oxidation during the latest Permian might have been important, another factor which should have contributed to a more gradual lowering of atmospheric oxygen during the mid-to-late Permian, is the general trend towards greater continental aridity, resulting in a lowering of worldwide organic matter burial. Burial of organic matter in sediments represents net photosynthesis and net oxygen production; thus, any large drop in worldwide organic burial should result in a drop in atmospheric O_2.

Because of the rise of vascular land plants and the deposition of their remains in terrestrial coal swamps and, by means of river transport, in the oceans, there was a considerable increase of worldwide organic matter deposition during the Carboniferous and Permian periods compared with earlier times. This conclusion is based on independent modelling of the carbon and sulphur isotope composition of marine sediments[4–6] and of the relative abundance of different types of sedimentary rocks[7]. (Isotope changes resulting from burial of organic carbon on land are sensed by the oceans by means of carbon transport to the sea by rivers and the atmosphere.)

One can combine organic burial rates with various assumptions about organic weathering to calculate changes in the level of atmospheric oxygen. The figure shows an example for the late Palaeozoic/early Mesozoic, based on the rock-abundance model[7]. Note the high sensitivity of atmospheric O_2 concentration to the value assumed for the average total organic carbon concentration in coal-basin sediments. A reasonable average value for the concentration of total organic carbon, present both as coal plus



Concentration of atmospheric oxygen as a function of geological age calculated via a sedimentary rock abundance model (adapted from ref. 7). Different curves are for the indicated average percentage of organic carbon in coal basin sediments, and the Permian/Triassic boundary is indicated by the vertical line marked P/Tr. The model is based on the relative proportions of sedimentary clastic rocks of this age that are present as coal basin sediments[8], reasonable assumptions regarding original worldwide sedimentation rates[7], the estimated range in the content of coal plus non-economic disseminated organic matter in coal-bearing sands and shales[5,9], and the average organic carbon content of marine sediments[8]. Organic burial rates, on which much of the oxygen results depend, agree with those calculated from independent models based on the use of carbon isotopes.

disseminated non-economic organic matter, in coal-basin sediments is about 2.5 per cent C. Thus, excessive burial of organic matter during the Carboniferous and early Permian may have resulted in elevated levels of atmospheric oxygen.

Later in the Permian, burial of organic matter on land declined, probably because of increasing aridity accompanied by less extensive swamp formation. (The preservation of organic matter on the continents is dependent on its deposition in standing water, such as in lakes and swamps, where the material is protected against oxidative destruction.) Organic-rich swamp and lake sediments were replaced by organic-poor continental red beds[8]. Red beds contain very little organic matter because they are formed in well-drained, fully aerated environments. Greater aridity should also have resulted in decreased transport of organic matter by rivers to the oceans because of both lower biological production on land and decreased river flow. Because of lower sea level at that time, increased sedimentation occurred on the continents at the expense of marine sedimentation, which also resulted in decreased organic burial because marine sediments are considerably higher in organic matter than red beds. The emerging continents plus increasing aridity must have involved a drop in worldwide sedimentary organic matter burial and worldwide oxygen production. Because of continued uptake of O_2 by the weathering of pre-existing organic-rich rocks, this drop probably also resulted in a drop in the level of atmospheric oxygen as shown in the figure.

I therefore conclude that a primary factor in causing a drop in both the $^{13}C/^{12}C$ ratio of sea water and the concentration of atmospheric oxygen during the Permian was the drying up of the continents. It is not clear what brought about this climate change.

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Malarial proteinase?

SIR—The DNA sequence encoding a major blood stage antigen of *Plasmodium falciparum* has recently been determined[1,2]. This sequence codes for a protein (relative molecular mass 111,000) of over 980 amino acids which is found abundantly in the parasitophorous vacuole of the erythrocyte stage and is processed to yield peptides at the merozoite release and reinvasion stage. Much interest has been focused on this antigen as a possible source of a vaccine against malaria as it is found to induce limited immunity in monkeys[3]. The function of the protein is unknown but using homology search[4] and multiple alignment software[5] we have now found that it shows weak but significant similarity to the cathepsin L, B and H group of cysteine proteinases.

Similarity among the cysteine proteinases is concentrated in two regions around the active site of the proteins; it is in these regions that the *Plasmodium* sequence shows greatest similarity. The figure shows an alignment of a diverse group of cysteine proteinases with the *Plasmodium* sequence around these two active-site regions. The putative active-site cysteine and histidine residues are highlighted. Outside these two active-site regions, the similarity between the different proteins is weak overall.

Dr Yongyuth Yuthavong (Mahidol University, Bangkok) has pointed out to us that proteases are known to be of major importance in the life cycle of *Plasmodium*, either for the degradation of haemoglobin during the erythrocyte stage, or for the cleavage of cell-surface proteins during the merozoite stage. Furthermore, a variety of protease inhibitors, including ones specific for cathepsin L, interrupt the normal life cycle of the parasite[6]. The sequence similarity reported here strongly suggests that the 111-K antigen, or a cleavage fragment of it, is a cysteine proteinase, making an already important protein even more interesting.

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Barley aleurain	..157aa	PVKNQAHCGSCWIFSTTGALEAA	..123aa..	DDVNHAVLAVGYG----V-ENGVPYWLIKNSWGADWGDNGYFK
Papain *(Carica papaya)*	..137aa	PVKNQGSCGSCWAFSAVVTIEGI	..118aa..	NKVDHAVAAVGYG----------PNYILIKNSWGTGWGENGYIR
Rat cathepsin H	..128aa	PVKNQGACGSCWIFSTTGALESA	..125aa..	DKVNHAVLAVGYG----E-QNGLLYWIVKNSWGSNWGNNGYFL
Rat cathepsin L	..127aa	PVKNQGQCGSCWAFSASGCLEGQ	..122aa..	KDLDHGVLVVGYGYEGTD-SNKDKYWLVKNSWGKEWGMDGYIK
Slime mould cathepsin	..131aa	PVKNQGQCGSCWSFSTTGNVEGQ	..128aa..	NSLDHGILIVGYSAKNTIFRKNMPYWIVKNSWGADWGEQGYIY
Plasmodium 111K antigen	..577aa	QVEDQGNCDTSWIFASKYHLETI	..150aa..	DTADHAVNIVGYGNYVNSEGEKKSYWIVRNSWGPYWGDEGYFK

Residues identical across all six sequences are indicated by (•); positions where four sequences exactly match the *Plasmodium* sequence are indicated by (–); positions showing only conservative[5] amino-acid replacements are indicated by (·), and the two putative active-site residues are indicated by (!).

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Trouble in scholarland

David Joravsky

Intellectual Compromise: The Bottom Line. By Michael T. Ghiselin. *Paragon House, 90 Fifth Avenue, New York, New York 10011, USA: 1989. Pp. 226. $24.95.*

MICHAEL Ghiselin has bared his soul in a protracted grumble against the world's affronts to virtuous scholars like himself. One's first impulse is to turn away in embarrassed silence, but the author's eminence — he is an outstanding biologist and historian of darwinism — demands serious consideration, and there are in fact some significant ideas within this tedious flow of basic truths that the dominant fools, rascals, hypocrites and liars of the academic world refuse to accept. "Liar" is Ghiselin's favourite accusation; I cannot remember hearing the epithet used so frequently or so loosely by anyone older than twelve. But I will pass over that mannerism and concentrate on the author's reasoning.

Ghiselin calls his book "social criticism", invoking as his models Tocqueville, Taine and Veblen. He confesses that he cannot truly imitate them, since his "intellectual roots are in zoology, not political science". But economics is the key to understanding human society, and economics is "a branch of biology in a broad sense", and natural selection is the *passe-partout* of biology. So an evolutionary taxonomist who has studied some economics is sufficiently equipped to diagnose the ills that afflict — well, maybe not society at large, which appears only briefly in Ghiselin's complaint, but certainly the learned part of it, which shares the common appetite for the trashy commodities that offend the author's soul.

Fraud in science

For example? The US system of distributing research funds and choosing articles for publication, which fosters mediocrity, trash and outright fraud in science. For *particular* example? I could find no actual specimens displayed in this book, which is full of irascible snorts and grim muttering about bad science but is curiously shy of providing illustrative instances. Ghiselin's closest approach is in reporting a test performed by Peters and Cecci. These authors made typed copies of articles in psychological journals, inscribed John Doe names with Upson Downs institutional affiliations, and resubmitted them to the same journals that had published them with known names and respectable affiliations. In only rare instances did editors or reviewers notice the fraud, reviewers nearly always finding substantive or "methodological" reasons to reject. Does this reveal something about psychology, or about all the scientific disciplines? Ghiselin assumes the latter, without any attempt to prove the point. I kept expecting a polemical exposé of mediocrity and trash in the biological sciences, especially in those areas where Ghiselin complains of trendy overfunding. But I found only smoky grumbling, no fiery illumination.

So Ghiselin's plea for good honest science in place of dishonest trash remains obscure. And his grand vision of biology and economics? Ghiselin is intent on persuading us that the historical approach to all living things is essential to biology, which thus becomes the instructor science for economics and human history. He provides brief reviews of some divisions among evolutionary biologists concerning the application of an historical viewpoint in their field, informing us of his major disagreements with G.G. Simpson, Stephen Gould, and even with the revered Ernst Mayr, who saved evolutionary biology at Harvard from destruction by molecular reductionists. (Mayr grates on Ghiselin by denying that there are laws of evolution in the same sense as there are laws of mechanical motion.) In other universities — my own, for example — the destructive reductionists have triumphed, and I looked eagerly for Ghiselin to dissect their stunted vision of the living world. Instead he tells all too briefly and enigmatically of his sympathetic interest in molecular biology, mentioning the grants he has received to work out its relevance to systematics. I was left wondering what inward division or restraining caution kept him from telling off representatives of the "nonhistorical, experimentalist ideology" that presses evolutionary studies towards extinction.

Puzzlement vanishes when Ghiselin comes to economics and human history. With clichés and caricatures he bounds over "Marxism", "historicism" and "cultural evolution", to reach a grand explanatory scheme that has somehow been overlooked by specialists in the disciplines of history and economics. Natural selection, supplemented by the economic principle of marginal utility, explains all. Reproductive success or failure of the species is the "bottom line", and marginal utility accounts for the human choices that will ultimately determine that success or failure. This simple formula enables Ghiselin to present his personal tastes as biological imperatives of reproductive success for the human species. It exempts him from the economist's entanglement in complex calculations of possible choices and results, and from the common historian's entrapment in endless interplay between the imagined past and the real past, not to mention the unknown future. Ghiselin is above all that. He offers brief synopses of his preferred solutions to disputed issues in biological evolution, brushes aside disciplined studies of human affairs in favour of global declarations about marginal utility, and is free to put the stamp of science on the intuitive judgements that have been shaped by personal experience of life, mainly academic, in the United States.

Generalities

Such judgements form the bulk of this sermonizing book, a tedious collection of banalities and dubious generalities concerning science and life, enlivened by autobiographical touches here and there — why the author moved from the revolting university setting to a civilized museum, how his book on the economy of nature was misinterpreted, how, "in presenting talks to academic audiences I have been accused of corrupting the youth — a charge that has also been leveled against people like Socrates, Jesus and Machiavelli". Such colourful accents are unfortunately rare: bromidic grey prevails. The reader who fears that I am being unfair can try these specimens: "Science invents theories and tests them by gathering data, and both of these activities are indispensable." "Prizes are one way of rewarding people for good work." "Scientists ought to be recruited from the ranks of the honest." "The leading scholars tend to be associated with the leading universities." "What academia really wants is a product that sells and gets no complaints from the customer."

The last generality is part of Ghiselin's central complaint against the academic world that shaped his mind and came to disgust him. One could as easily argue the opposite — that academia has standards other than customer satisfaction — and Ghiselin intermittently does so. He goes back and forth between the complaint that shoddy goods dominate the market-place of academic thought and the confidence that "a good theory drives its inferior competitors out of the market". Examples can be given to support either side of the argument, depending on one's tastes and preferences. Ghiselin, as I have said, does not provide examples to show what he means by the shoddy goods that dominate the market-place in biological science, but he becomes specific, and occasionally abusive, about philosophers and historians of science. Thus, Feyerabend's rule that "anything goes" in creative science "was just the sort of attitude that was popular with the Telegraph Avenue drug culture of accursed memory". And he calls William Coleman and Garland Allen

spinners of marxist fairy tales for showing how Haeckel's speculations were at odds with the chief trends in biological science.

That last bit of polemics is more revealing than Ghiselin intended. Coleman, who was president of the history of science society until his death from leukaemia last year, was as far as one can imagine from any form of confessional marxism, and equally far from the habit of using 'marxist' as a dismissive epithet rather than an eponym, like 'darwinian', for one of the most important trends in modern thought. As for fairy tales in place of painful reality, one must ask why Ghiselin is so anxious to rescue Haeckel from his reputation as an irresponsible spinner of evolutionary explanations for everything. Coleman and Allen annoyed Ghiselin by showing how comparative embryology was pushed aside by experimental embryology at the turn of the century, with far more serious consequences than the decline of esteem for Haeckel. What Ghiselin angrily calls "nonhistorical, experimentalist ideology" gathered force in departments of biology until the very existence of evolutionary studies was threatened. Ghiselin has reason to feel dismayed, but hardly to jump on the historians who have told what happened, especially not on such scholars as Coleman and Allen. Their histories of biology are as respectful as he is of systematics and evolutionary studies, including comparative embryology. Once again, Ghiselin fails to engage in polemics with biologists who promote molecular reductionism, and vents spleen on individuals who are actually his comrades on fundamental issues.

As an historian of left-wing movements, I see here a depressingly familiar pattern. When the left is in greatest peril, pressed towards extinction through declining numbers, internal squabbling begins to prevail over the criticism of power that is the left's reason for being. Ghiselin will no doubt be startled to find himself identified with the left, if only in the little world of biologists. I intend this as a compliment, and I offer as conclusive evidence his praise for "The Commoditization of Science" by Lewontin and Levins. (See their collection of marxist essays. *The Dialectical Biologist.*) Indeed, I would endorse his praise of that essay, which lays out the main problem that bothers Ghiselin, and does so more effectively.

Let me state the problem as I perceive it. Modern society, whether in its capitalist or its socialist forms, tends to change scientific research into the production of commodities for sale rather than the quest for truthful knowledge, not to speak of wisdom. Different strata of scientists have been affected in different degrees, as Lewontin and Levins effectively point out. (Ghiselin's alternative description of the strata — rascals, fools, hypocrites, liars and honest souls — is less useful.) Although most US scientists are directly involved in commodity production, working either as hired hands or as bosses in the service of profit rather than truth, a minority cling to the intellectual autonomy of the traditional artisan or the traditional client of royal patrons. But intellectual autonomy tends to be undermined even within that minority by several forces, the most obvious of which is the bureaucratization of patronage, that is, the modern system of grant-getting. It tends to reward those who seek to satisfy a bureaucratic hierarchy, rather than those who seek truth, not to speak of wisdom.

I have deliberately injected troublesome concepts — intellectual autonomy, truth, wisdom — for a life in science is supposed to approach those priceless goals, and the effort to discuss basic problems of science without reference to them confuses rather than clarifies the issues. Nor can the discussion be limited to abstract philosophical analysis. Anxiety about the commoditization of science is part of a pervasive worry in all areas of modern life. Modernity requires and at the same time rebukes the autonomous mind, the self that would truly know how it stands in relation to the world at large. Variations on that theme appear not only in *The Communist Manifesto*, where Lewontin and Levins found it, but also in Spinoza and Rousseau and Schiller, where Marx found it. A roll-call of famous thinkers who have developed striking versions of it would be impossibly long.

Let me note merely that scientists are quite mistaken if they regard this as a problem only for philosophers or imaginative writers. When Einstein declared that most scientists are imprisoned in the "traditions of the herd" (see Einstein-Born *Briefwechsel* 203 (1969)) by their training in "mechanized and specialized thinking", he was echoing the Nietzschean version of the anxious theme. It is far too complex to be dealt with adequately here, but it needs to be mentioned, for the greatest deficiency of Ghiselin's book is its unawareness of the tradition within which his questing mind is thrashing about. I urge him to read Max Weber's great lecture, "Science as a Vocation", to get a sense of the tensions that moved him to write this book. □

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Round in circles

Lee A. Segel

Free Energy Transduction and Biochemical Cycle Kinetics. By Terrell L. Hill. *Springer-Verlag: 1989. Pp.119. Pbk £19.*

THERMODYNAMICS is a difficult subject for many scientists, including myself. The difficulty lies in the fact that thorough understanding requires full intuitive appreciation of macroscopic entities (such as entropy and various forms of free energy) and their relationships. Yet these relationships can only be derived in an entirely convincing fashion by probabilistic analysis of underlying kinetic models. The subtlety of the analysis required is attested to by the continuing generation of important new results, even for what seem superficially to be relatively simple situations.

For decades, Terrell Hill has been a major figure in such 'thermostatistical' research (although ugly, 'energo-statistical' might be a better neologism, for at least in the biological sciences energy transduction and dissipation often lie at the centre of attention, not temperature changes). The book under review is essentially an abridged, simplified and updated version of his *Free Energy Transduction in Biology* (Academic, 1977), which in turn was primarily a distillation of his own research. The present title is more accurate, for a considerable amount of material on cycle kinetics is included. All but the simplest biochemical reaction schemes contain cycles (with accompanying constraints imposed by the requirement of microscopic reversibility).

The basic model treated consists of a number of discrete states with assigned constant transition probabilities per unit time between every pair of states, in both directions. The model can be represented by a diagram (or 'graph' in the more modern mathematical sense) consisting of vertices corresponding to the states and arrows corresponding to the transitions allowed. Many important problems arise, such as determining efficient and intuitively satisfying ways to calculate the rate at which a given cycle on the diagram is traversed, or the mean number of cycles before a final absorbing state is attained. These matters are purely probabilistic. Thermodynamics enters when free-energy levels are calculated for the various states, and fluxes are related to forces that are proportional to differences in free energy.

The book is clearly written, with a commendable plan of illustrating concepts by detailed treatment of carefully selected examples. Although no exercises are provided, this slim and interesting volume indeed fulfils its goal "to be a textbook for a class or for self-study". □

Lee A. Segel is in the Department of Applied Mathematics and Computer Science, Weizmann Institute, Rehovot, Israel.

The natural diagnosis

Roy Porter

Forces of Change: Why We Are the Way We Are Now. By Henry Hobhouse *Sidgwick and Jackson: 1989. Pp. 264. £17.95.*

IN HIS best-selling *The Rise and Fall of the Great Powers,* Paul Kennedy offered a historian's reading of 'why we are the way we are now'. The global dynamics of East and West, of First, Second and Third Worlds, depend in the short run upon military strike power, but fundamentally upon political will and industrial strength. The world's future hangs upon superpower rivalry.

In *Forces of Change*, Henry Hobhouse offers a second opinion upon our problems and prospects. His diagnosis diverges radically. Mankind's true destiny, he argues, may have little to do with such strategic rivalries — he barely conceals his contempt for politicians and all the politicians' men. Rather, the true dynamics of history — too often ignored by blinkered historians — are biological and ecological. Spain conquered the Indies not by superior fire- or faith-power, but because it had smallpox on its side. The United States became the world's top dog not because of democracy and freedom, but because of infinite natural resources, above all, fertile land. The 'forces of change' are, at root, biological.

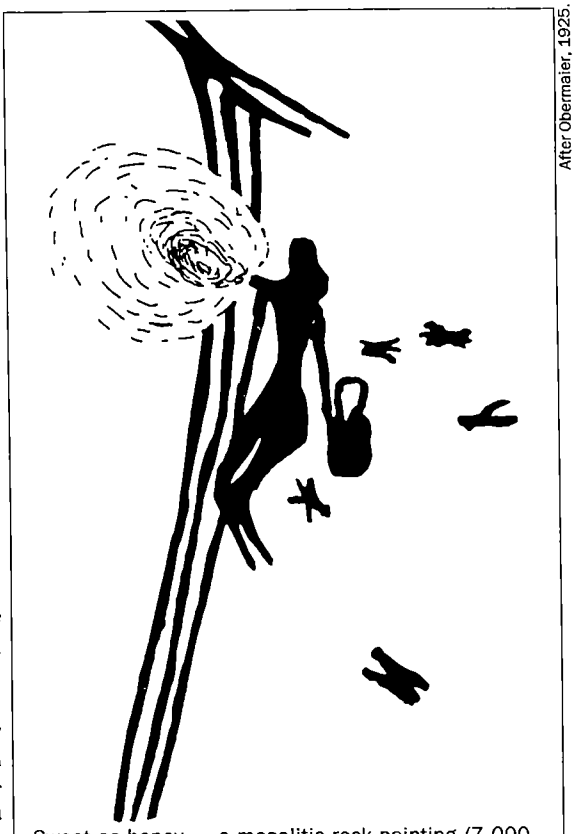
The natural history of man, he contends, results from a triangle of forces: population growth, food output, and disease. Around 1800, the pioneer demographer, Thomas Malthus, argued that progress was an impossibility, because man's propensity to breed would always outrun food supply: famine, pestilence or war would inevitably ensue. Have we at last succeeded in escaping these malthusian 'checks'? That is the question.

Superficially, at least, the answer might seem to be yes. Medicine is disarming disease. By consequence, global population has doubled twice this century and will do so again before 2030. War has barely culled these numbers, and only local, not global, famine has intervened. This is because food yields continue to rise phenomenally where efficient husbandry, science and technology, pesticides and the 'green revolution' have been shrewdly used. Future biotechnological prospects look rosy.

So is Malthus defeated? Hobhouse's answer — in a book by turns angry and witty — is basically no. Certainly, we can multiply population, but on most continents all we do is multiply misery, as peasant subsistence farming disintegrates and the despairing masses flock to nightmare cities. Malthusian checks themselves may well have the last laugh with AIDS. And, of course, so-called progress is precipitating a new range of checks unknown to Malthus — the greenhouse effect, acid rain and the destruction of the ozone layer. Recognized all too late in the day, environmental pollution is perhaps already irreversible.

So far, so familiar. Hobhouse argues his brief with energy, combining historical vision with an expert grasp of agricultural business; but the basic analysis (condition chronic, possibly terminal) is hardly news. What rescues this diagnosis from the commonplace is its plain-man's trenchant realism, dismissive of pieties, platitudes and pie-in-the-sky.

Almost without exception, planners' attempts to cure by *diktat* the problems of population and hunger have proved counterproductive. From Stalin's Russia to Ethiopia, state socialism has been an eco-disaster. Hobhouse shows no more sympathy for high-minded movements like Greenpeace (sentimental, self-indulgent hot air), or international aid agencies, which palliate today's problems only to worsen tomorrow's. Native ways were wiser than Western do-gooding: better to have infanticide and high perinatal mortality on the savannah than surplus millions rotting in refugee camps. At least such folkways weeded out the 'unfit'.



After Obermaier, 1925.

Sweet as honey — a mesolitic rock painting (7,000–4,000 BC) from Cuevas de la Araña, Bicorp, Spain, depicting a woman with a basket gathering wild honey from a hive at the top of a tree. The picture is taken from *Women in Prehistory* by Margaret Ehrenberg, published by the British Museum.

And here, in this tell-tale term, harking back to social darwinism, we have the clue to Hobhouse's own nostrums. Like all other creatures, humans flourish when their habits 'fit' a particular environmental niche. The neo-Europes, New Zealand above all, have achieved a healthy 'fit' between human demands and natural resources. Japan's success is due more to its super-efficient land husbandry than to technological wizardry. In other words, Hobhouse sets store by a kind of territorial imperative: every population must carve out its own *modus vivendi* with nature, and then protect that survival strategy.

This requires a healthy individualism. Paternalism and egalitarianism are out in Hobhouse's tough-minded neo-darwinism: the idea of free health care at the point of service is a nonsense, he asides. We mistakenly look to leaders for magical answers, he says. Vest (some) faith in science, but none in politicians, for know-all, vote-seeking governments generally turn out to be environmental hazards.

Hobhouse's commitment to nature's laws, to knowledge and enterprise, and his hatred of humbug has an attractive ring — as, of course, had Victorian social darwinism. But, for all its trumpeted 'realism', it has its own fatal blind-spot. For in the modern world you cannot divorce nature from politics. It is all very well to assert that the real problem of South Africa is not apartheid but the Bantu population explosion and consequent agricultural under-capitalization; but it takes a certain wilful myopia to treat these as unconnected. Trust to enterprise, Hobhouse urges, and you minimize waste. Maybe. But maybe you also end up, as he himself admits, with Thatcher's Britain disgorging vast quantities of untreated sewage and pollutants into the North Sea (and surely that is waste, by anybody's definition, not to say ecologically stupid).

It is disturbing to find a born-again darwinian convinced that enlightened competitive individualism will rectify today's ecological imbalances. For was it not these very 'forces of change' that first created the problems? Yet in these paradoxes lies the genuine stimulus of Hobhouse's spiky and often opinionated analysis. By tilting at conventional wisdoms, he forces us to think hard and deep about our true relationship with our planet. □

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Honesty's the best policy

J. L. Heilbron

Science à la Mode: Physical Fashions and Fictions. By Tony Rothman. *Princeton University Press: 1989. Pp.207. $19.95, £12.50.*

IN THE middle of the eighteenth century, the Berlin Academy of Sciences advertised an essay competition on the question, "Is it useful for men to be deceived?". In so far as they have a common theme, the six essays served up here under the opaque title *Science à la Mode* may be regarded as answers to this enduring question.

The essays, four of which have been published previously, consider modern cosmology (twice), geodesics, entropy, nuclear winter and the career of Evariste Galois. The first four are mostly high-level vulgarization. The last two criticize scientists who play to the public, bend to politics or write poor history. Even when popularizing, however, Rothman stops to admonish his colleagues (he is a cosmologist) for coveting their own hypotheses and evolutionary biologists for misusing ideas (the doctrine of entropy) they do not understand. Many examples of both sorts of self-deception come to mind. The tychonic system, the theory of phlogiston, the electromagnetic ether and light conceived as waves or particles, or both, or neither, have had their over-fond exponents; and physicists themselves have helped to cheapen their ideas by advertising parallels between quantum theory, depth psychology and Eastern mysticism.

It is not self-deception, however, but wilful misrepresentation of technical concepts or conscious relaxation of professional standards against which Rothman aims his two most useful essays. One records attempts to evaluate the claims made by Carl Sagan's group about the likelihood that man will exterminate himself and herself by throwing up enough smoke in a nuclear war to shut out the light of the Sun. Rothman faults the trumpeters of this dusty eschatology on two counts: their shaky numbers and hypotheses do not authorize the conclusion that we can do for ourselves what Alvarez's meteor may have done for the dinosaurs; and, by shifting attention from the colossal number of direct and immediate casualties from any serious nuclear exchange, they encourage pursuit of technical palliatives to the twilight rather than urgent efforts to remove the arsenals that might cause it. Rothman has no patience with colleagues who doubted the reliability of the wintry calculations but refused to say so, thinking that the prospect of the end of the human race might promote the cause of disarmament. Here the problem of the utility of deception re-presents itself. Rothman's solution is that honesty is the best policy.

The essay on Galois corrects the story commonly told by scientists of a young unappreciated genius, persecuted for his political opinions and killed in a duel arranged by police agents. Galois was a trouble maker, his genius was recognized, and he died for love not revolution. (The historian could supply hundreds of similar cases in which scientists have neither tried nor wanted to get the story straight; a short list appears in Franz Stulhofer's recent book, *Lohn und Strafe in der Wissenschaft: Naturforscher im Urteil der Geschichte*, published by Böhlau, Vienna, in 1987.) Rothman condemns the misrepresenters of Galois for suspending their usual standards of research and for deceiving their students in order to parade a picture of brilliance destroyed by functionaries dumb to the calls of genius. Thence it is an easy step to the caricature that everyone is wrong and stupid but the creative scientist. The caricature may have its tactical uses, but it is bad strategy. Rothman rightly condemns it. "This is a presumption of the highest arrogance [he writes]. Scientists should not be so enamored of themselves." □

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Cellular update

Ron Laskey

Molecular Biology of the Cell, 2nd Edn. By Bruce Alberts, Dennis Bray, Julian Lewis, Martin Raff, Keith Roberts and James D. Watson. *Garland:1989. Pp. 1218 + index. Hbk $51.95; pbk £24.95.*
The Problems Book. By John Wilson and Tim Hunt. *Garland:1989. Pp.354 + index. Pbk $14.95, £8.*

THE first edition of *Molecular Biology of the Cell* appeared in 1983 and marked a milestone in the publication of textbooks in the life sciences. In order to cover the full range of cell biology, a team of authors was involved, but the material was integrated in such a way that it read as the work of a single hand. The book's success was immediate, and its appeal — to novice students, university teachers and specialized researchers — remarkably broad.

But six years is a long time, especially in such a fast-moving field as cell biology. So for some time there has been an impatient clamour for a second edition, and there is no doubt that an up-dated version was worth writing. The only question left for a reviewer is "Does it match the standard of its predecessor?". In my opinion the answer is "No". It doesn't match the first edition. It surpasses it.

A first glance at the "Contents in Brief" could mislead the reader into thinking that the second edition is almost the same as the first. There is only one entirely new chapter (an excellent one on cancer) and the old chapter on the cell nucleus has been split in two to generate a separate discussion of control of gene expression. Apart from these changes and some reordering of the contents, the book at first sight looks much the same as before.

It is only on starting to read that one realizes how radical the revision has been. Overhaul has not simply involved the addition of new examples, but thorough restructuring of the conceptual framework. In all those cases I can judge, the improvement is substantial. The illustrations are another area of improvement, the explanatory diagrams and dramatic micrographs working in harmony with the text. Here is a commendably up-to-date and accurate account of the best of cell biology.

The advent of the accompanying problems book by John Wilson and Tim Hunt is a further bonus, especially for students. The book follows the main text and allows readers to test their comprehension of facts and concepts through several levels of question. The questions range from filling in the blank in a sentence or identifying a statement as true or false, to solving complex problems, which are often based on real experiments from the literature.

One matter continues to puzzle me. How have the authors managed to pack in so much new information (the developments in cell-cycle control and many other topics besides)? The length of the book is much the same as before, and there has been no sacrifice of the lucid, accessible style that marked the first edition.

So is the book really perfect? No, of course not. It is possible to identify a few niggles, such as a wrong page number in the index and oversimplified 'true or false' questions in *The Problems Book*. There are also minor points on which I disagree with the authors' emphasis or interpretation. But the overall standard of the new edition is so overwhelmingly excellent that I have no reservations in pronouncing it the best book of its kind — another milestone in biological education. □

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• An English translation of Anatole Abragam's autobiography, *Time Reversal*, is being published by Oxford University Press, priced at £25, with a release date at the end of August. The original French version of the book was reviewed in *Nature* last year. (See *Nature* **333**, 126; 1988).

The allosteric transition of glycogen phosphorylase

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The crystal structure of R-state glycogen phosphorylase *b* has been determined at 2.9 Å resolution. A comparison of T-state and R-state structures of the enzyme explains its cooperative behaviour on ligand binding and the allosteric regulation of its activity. Communication between catalytic sites of the dimer is provided by a change in packing geometry of two helices linking each site with the subunit interface. Activation by AMP or by phosphorylation results in a quaternary conformational change that switches these two helices into the R-state conformation.

ALLOSTERIC proteins are characterized by their possession of distinct binding sites whose affinities for certain ligands depend on indirect interactions between these sites. These proteins have vital roles in metabolic regulation and receptor response. Glycogen phosphorylase *b* was the first allosteric enzyme to be isolated and analysed in detail[1]. The enzyme catalyses the first step in the intracellular degradation of glycogen,

$$(\alpha\text{-1,4-glucoside})_n + P_i \rightleftharpoons (\alpha\text{-1,4-glucoside})_{n-1} + \alpha\text{-glucose-1-P}$$

and is regulated both by allosteric interactions and reversible phosphorylation[2]. This regulation can be understood in terms of an equilibrium between several conformational states[3], ranging from a T-state with low affinity to an R-state with high affinity for substrate and activators, in which quaternary structure subunit interactions and tertiary structures are modulated by ligand binding. Phosphorylase *b*, the unphosphorylated form of the enzyme, is dependent on AMP for activity, and this activity is inhibited by glucose-6-phosphate(G6P) and ATP (Fig. 1). The enzyme shows homotropic cooperative effects (that is, interactions between identical ligands) for binding of substrates and AMP (Hill coefficients 2 and 1.4, respectively[4]) as well as heterotropic effects (that is, interactions between different ligands). The enzyme, for example, shows a 15-fold increase in affinity for phosphate as the concentration of AMP is increased from 0.015 to 0.5 mM (ref. 5). Physiological activation by hormonal or neuronal signals stimulates the conversion of phosphorylase *b* (GP*b*) to activated phosphorylase *a* (GP*a*) by kinase-catalysed phosphorylation of a single serine residue, Ser 14.

In the absence of effectors, GP*b* is a dimer composed of two identical subunits each having a relative molecular mass of 97,434. Activation by AMP or phosphorylation produces a change in the oligomeric state of the enzyme: it becomes largely tetrameric. Tetramers have only 12–33% of the full phosphorylase activity, but may be dissociated by glycogen or oligosaccharides to give fully active dimers[6–8]. A substantial amount of phosphorylase *in vivo* is bound to glycogen particles[9–11], and the dimer is considered to be the physiologically active form of the enzyme[2].

High-resolution X-ray structures have been solved for T-state GP*b* (in the presence of a weak activator, IMP[4,12]), and T-state GP*a* (in the presence of an inhibitor, glucose[13,14]) (Fig. 1). Comparison of both structures[15] revealed the conformational changes resulting from Ser-14 phosphorylation. Communication of these changes to the catalytic site was restricted by crystal lattice forces that restrain both structures in the T-state. Here we report the structure of R-state GP*b* determined by molecular replacement methods, using as the search object the refined (1.9 Å resolution) T-state model of GP*b* (ref. 16, and K. R. Acharya *et al.*, unpublished results). As reported previously[17–19], monoclinic crystals of GP*a*, GP*b* or GP*b* bound with AMP can be obtained in the presence of ammonium sulphate (1 M). Under these conditions, GP*b* displays R-state characteristics such as catalytic activity, enhanced affinity for AMP, a reduced Hill coefficient, reversal of inhibition by G6P and glucose[20,21], and the association of dimers to form tetramers. Crystallographic studies show that sulphate mimics phosphate by binding to the catalytic site, the AMP effector site and the Ser-phosphate site, resulting in localized changes in tertiary structure. In the transition from the T- to the R-state, the two subunits of the functionally active dimer rotate about an axis perpendicular to the 2-fold symmetry axis to accommodate these changes in tertiary structure. The correlation between the tertiary and quaternary structure provides an explanation for the cooperativity of substrate and effector binding, and their mutual interdependence.

Structure determination

Crystals of rabbit muscle R-state GP*b* were obtained in ammonium sulphate (1 M) and β-glycerophosphate (10 mM, pH 7.5) as described[17,18]. Native data, to a resolution of 2.9 Å, were collected at the Synchrotron Radiation Source, Daresbury on an Arndt–Wonacott oscillation camera. Strong low-resolution reflections were recorded in a 3.5 Å data set, which was subsequently incorporated into the 2.9 Å data set (Table 1).

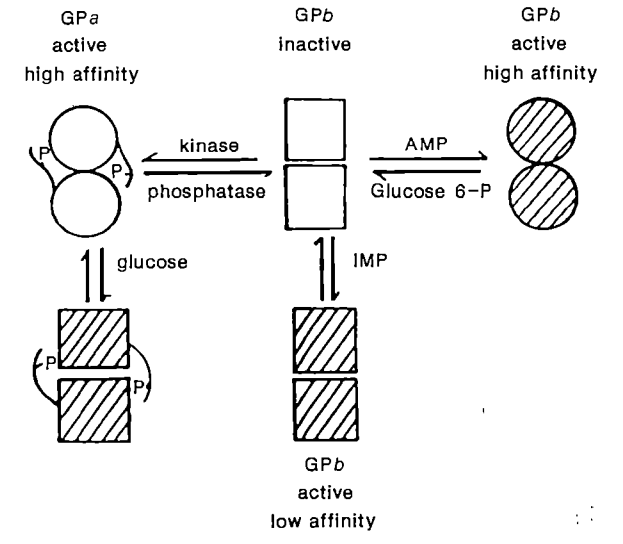

FIG. 1 A simplified representation of the allosteric and covalent activation of glycogen phosphorylase. T and R subunits are shown as squares and circles, respectively. The states referred to in the text are shaded.

The structure was determined by the molecular replacement method[22], using the T-state monomer as a search object. Rotational parameters for each subunit were determined, and the translation vectors relating crystallographic and non-crystallographic subunits obtained using the Crowther and Blow translation function[23]. Rigid body positional refinement, first with individual subunits and then with individual domains, was performed using the program COREL[24]. The resultant structure was subjected to a round of atomic positional refinement by the method of simulated annealing with the XPLOR program[25] using a CONVEX C210 supercomputer. Following convergence, a $2F_o-F_c$ electron density map was averaged according to the geometric relationships of the four subunits. The map obtained indicated new positions for parts of the structure which underwent large conformational changes. One subunit was manually rebuilt into the averaged density and a tetramer constructed from it. Least-squares refinement with energy restraints was then used. Non-crystallographic symmetry restraints were initially applied and gradually relaxed as the R-factor dropped. The final crystallographic R-value was 0.177.

Description of overall structure

Both R- and T-state structures possess a similar topology. The enzyme is an α-β protein with the polypeptide chain folded into two domains; the N-terminal domain of amino-acid residues 1–484 and the C-terminal domain of residues 485–842. In addition to the recognition site for the 5′-phosphate of pyridoxal phosphate (PLP)[26], the enzyme has three other recognition sites for the phosphate: (1) substrate (site ~5 Å from the cofactor phosphate); (2) AMP (site ~30 Å from the catalytic site); and (3) Ser-phosphate (site ~12 Å from the AMP site). Examination of the R-state structure shows that each of these three phosphate-recognition sites are occupied by sulphate (Fig. 2).

When two GP*b* dimers associate, the four subunits arrange in a planar array obeying molecular 222 symmetry (Fig. 2). The α-carbon (C_α) positions of all subunits deviate from the mean by a root-mean-square (r.m.s.) of 0.3 Å, indicating that the four subunits are similar within the limits of data precision. The interface that is formed because of the association of the dimers to tetramers comprises residues from the 'catalytic' face of the subunit so that access to the catalytic site is slightly impeded. The involvement of a key residue from the glycogen storage site (Glu 433)[27] in a hydrogen bond at this interface explains why glycogen promotes dissociation of tetramers to dimers. The details of the contacts at the tetramer interface will be described later. The subunit interface of the dimer is composed of two main contact regions occurring on opposite sides of the enzyme molecule (Fig. 3). One is formed between the cap′ region (residues 35′–46′), a loop connecting the α1′ and α2′ helices with the β7 strand, and the α2 helix (residues 47–78) of the opposite subunit (residues from the symmetry-related subunit are designated by primes). This interface includes the AMP and Ser-phosphate binding sites. The other contact is formed by the antiparallel association of the two symmetry-related tower helices, α7.

A least-squares superimposition[28] of the R-state subunit onto the T-state subunit reveals the extent of the tertiary conformational differences between the two states. The overall r.m.s. deviation in C_α positions is 1.3 Å, although that for a core of 80% of the C_α atoms is within 0.7 Å. Those regions that deviate significantly between the two structures are either important for the allosteric response, (the tower helix), or form part of the new tetramer interface (helices α12, α13 and α23–α27). Relative movements between the two domains do not occur.

An extensive quaternary structural change is observed such that after superimposition of one subunit of the T-state dimer onto a subunit of the R-state dimer, the r.m.s. deviation in C_α positions for the two dimers is 6 Å. This rearrangement consists of a 10° rotation of one subunit relative to the other about an axis that is approximately perpendicular to the 2-fold axis of the dimer and that intersects the dimer axis close to the region where the cap′ links to the α2 helix (Fig. 3). The rotation draws

TABLE 1 R-state GP*b* data collection, processing and refinement statistics

Crystal parameters
Space group $P2_1$ a=119.0 Å, b=190.0 Å, c=88.2 Å, β=109.35°
One tetramer, relative molecular mass of 390,000 per asymmetric unit

Data collection and processing parameters

Data set	No. crystals used	No. measurements	No. independent reflections	*R_{sym}	Percentage with $I>3\sigma_I$	Percentage complete
2.8 Å Native	10	86,341	59,294	0.12	64	81
3.5 Å Native	1	51,459	30,319	0.075	66	75
2.8 Å and 3.5 Å native	11	131,558	64,860	0.13	65	88
4.2 Å TAMM†	3	40,133	22,983	0.045	84	88

Refinement parameters
No. protein atoms=26,816 Number of sulphate atoms=60
No. observations, $F>\sigma_F$, 8.0–2.9 Å=57,293
‡R_{cryst}=0.177
r.m.s. deviation of bond length from ideality=0.018 Å
r.m.s. deviation of bond angles from ideality=4.0°
No water molecules were included in the refinement

Data were collected on the Wiggler station PX9.6 of the SERC Synchrotron Radiation Source, Daresbury, UK. The source was operated at an energy of 2 GeV and current ranging from 250 to 150 mA, wavelength=0.88 Å. Typical crystal dimensions were 1.0×0.5×0.3 mm³. Spindle oscillation range was 1.0° for the 2.8 Å data set and 2.0° for the 3.5 Å and 4.2 Å data sets. Films were scanned on a Joyce-Loebl Scandig 3 microdensitometer and the intensities integrated by profile fitting with a modified version (D. I. Stuart, unpublished data) of the MOSCO data processing program[45] using a PDP/11 computer. Interpack scaling, partial reflection summation and data reduction were performed by the AGROVATA and ROTAVATA programs, Daresbury CCP4 program suite. TAMM derivative crystals were prepared by soaking in a solution of TAMM (0.5 mM) for 3 h. The TAMM data set was scaled to the native data using an anisotropic temperature scaling function (program ANISOSC, CCP4 suite) giving a mean fractional isomorphous difference of 12.0%. The five major heavy atom sites, derived from a difference Fourier map calculated using the TAMM and native amplitudes with calculated phases, were consistent with those assigned earlier from a 6 Å difference Patterson map[46] and suggested binding of a tetramercurial compound. Four of these sites are associated with Cys 783, a cysteine that was reactive in the T-state structure[47]; a fifth site was to Cys 495 of the second subunit.

* $R_{sym}=\sum_i\sum_h|I(h)-I_i(h)|/\sum_i\sum_h I_i(h)$, where $I_i(h)$ is the ith measurement of reflection h and $I(h)$ is the mean of the intensity.

† TAMM, tetrakisacetylmercurialmethane

‡ $R_{cryst}=\sum_h|F_o-F_c|/\sum_h F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection h

the two subunits together at the cap′/α2-helix contact region by 1 Å and moves them apart at the tower–tower interface region by 3 Å. Details of the changes in subunit contacts are given in Table 2.

Structural changes at the tower–tower interface

The tower helix (residues 262–276) consists of a protuberance from the main body of the enzyme. It is linked to the preceding β-sheet, (residues 237–247) by a region of flexible polypeptide chain (residues 252–258). Residues 250–275 exist autonomously from their own subunit, making sparse interactions with it. The helix is anchored at its base by a short parallel β-sheet formed between residues 276–279 and 162–164. From here the polypeptide chain leads to the reverse loop of residues 281–287 (280s loop), which form a gate to the catalytic site and lead into the α8 helix (residues 289–314).

In the T-state, an interface is formed by the antiparallel association of the two tower helices, creating a cluster of hydrogen bonds between Asn 270 and Asn 274 and their symmetry-related counterparts (Fig. 4). The unfavourable packing angle of −20° (ref. 29) is achieved by unwinding and distortion of the helices between residues 270–273. Tyrosine 262 and Ile 263 pack against Pro 281′ and Tyr 280′ of the symmetry-related subunit, respectively. On transition to the R-state, the geometric disposition of the helices is dramatically altered following an increase in the inclination of the helix axes to an orthodox −80°. The tilting of the two helices brings Val 266, Ile 267 and Asn 270 into a nonpolar interaction with their symmetry equivalents on the opposite subunit. The helices slide relative to each other by two turns of helix. Tyrosine 262 and Ile 263 no longer pack against Pro 281′ and Tyr 280′.

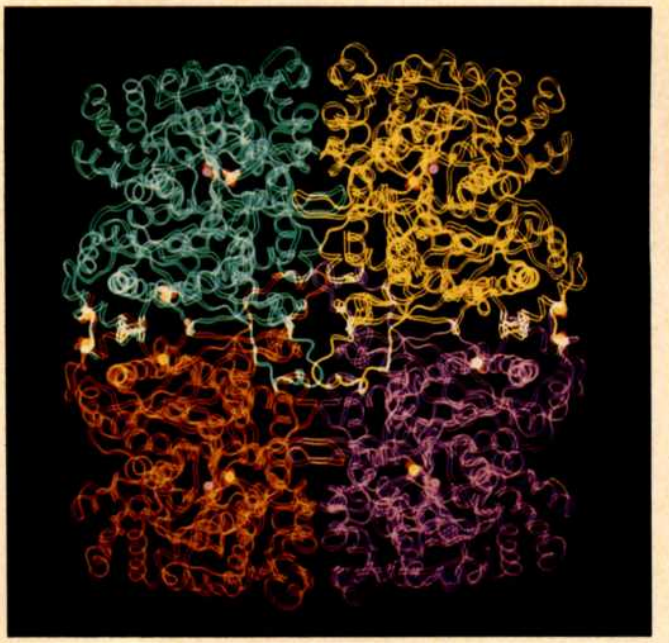

FIG. 2 Ribbon representation of C_α atom trace of the R-state GP*b* tetramer indicating the molecular 222 symmetry. Sulphate ions are shown in yellow and the phosphate of pyridoxal 5′-phosphate in purple. The dyad axis to the T-state GP*b* dimers is horizontal. The dimer interface formed by the association of the two dimers is vertical. This interface involves contacts between residues from the glycogen storage site (prominent loop representing the β15–β16 turn) and from the bundle of helices α23, α24, α26, residues 724–767.

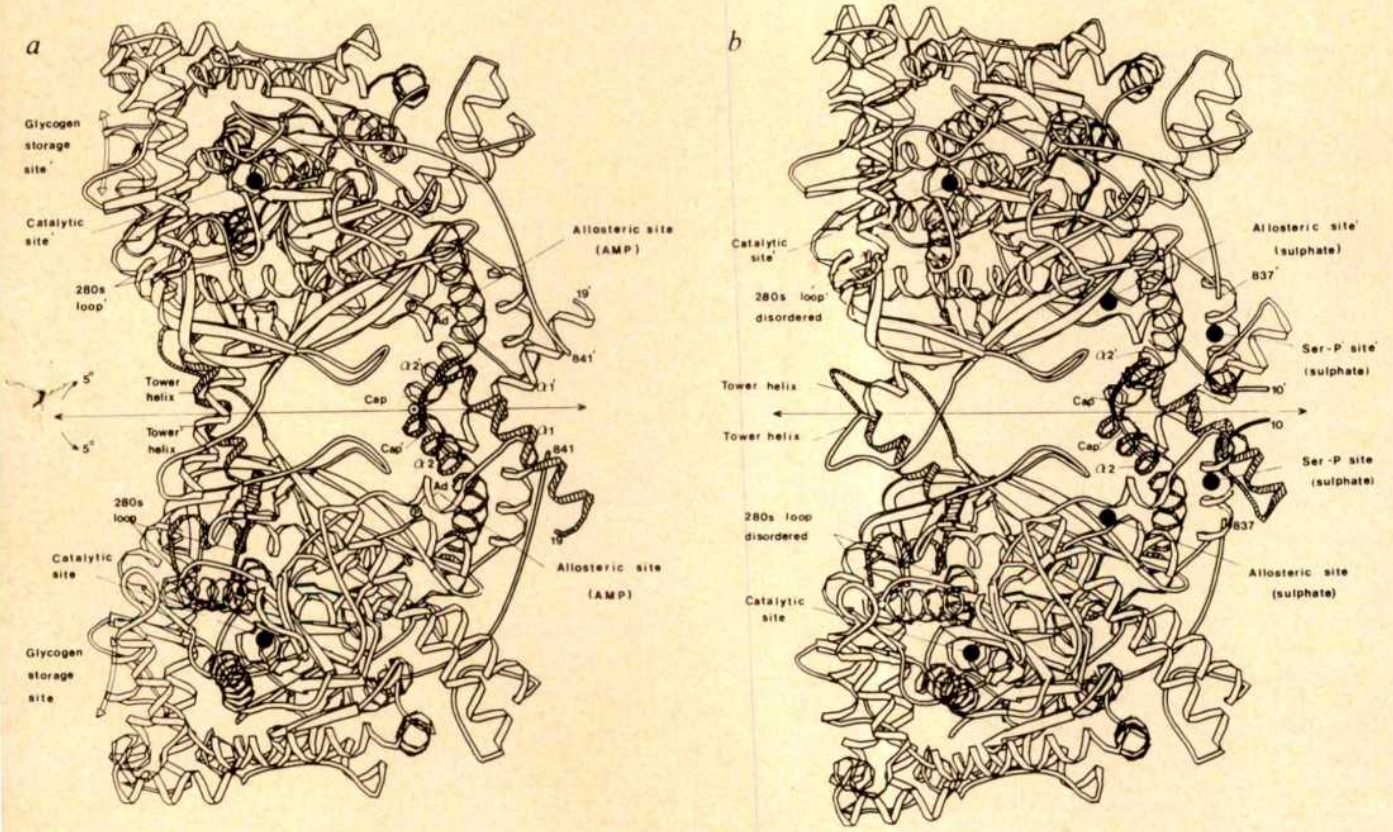

FIG. 3 Ribbon representation indicating the structural elements of: *a*, T-state, and *b*, R-state phosphorylase dimer, viewed perpendicular to the molecular dyad axes. The view is rotated 20° about the horizontal 2-fold axis of Fig. 2, and represents the yellow and purple subunits of Fig. 2. Structural elements α1–cap–α2, the tower helix-280s loop and the start of α8 are indicated by shading in the lower subunit. The allosteric effector site and Ser-phosphate site are situated at the cap′/α2 interface. The catalytic site is close to the 280s loop at the termination of the tower helix. The glycogen storage site, to which phosphorylase dimers are attached to glycogen particles *in vivo*, is situated on the 'catalytic face' of the molecule and forms a dimer–dimer contact in the tetramer. On transition to the R-state, each subunit rotates by 5° about an axis positioned close to the cap′/α2 interface (marked by ⊙ in Fig. 3*a*). Ribbon diagram by a program by J. B. Priestle.

The change in helical cross-over angle is a consequence of both a quaternary and a tertiary structural change, involving a 20° tilt of the helix as an approximate rigid body relative to the remainder of the enzyme. A superimposition of the R- and T-state tower helices gives an r.m.s. deviation of 2 Å in C_α positions. This superimposition shows that the largest difference occurs between residues 272–277, and because Val 278 of R- and T-state subunits superimpose, indicates that the change in tilt of the tower helices involves adjustments in main-chain torsion angles and hydrogen bonds within the last turn of the helix. This causes related changes in the hydrogen bond pattern of the β-sheet formed between residues 276–279 and 162-164. In response, residues 162–165 twist, displacing Ile 165 by 1.5 Å. This displacement directly perturbs Asn 133 and Pro 281, transmitting a structural change to the catalytic site (Fig. 5). Thus a conformational change of the tower helix mediates transmission of a conformational response from the subunit interface to the catalytic site.

Structural changes at the catalytic site

Numerous binding studies with substrates and substrate analogues in the T-state enzyme[30,31] have located the catalytic site ~15 Å below the molecular surface at the base of a narrow tunnel formed between the interface of the two domains, and close to the essential cofactor PLP. An inhibitory site situated at the entrance to the catalytic-site tunnel is composed of two hydrophobic residues, Phe 285 and Tyr 613. At high concentrations AMP, IMP and other related planar non-polar molecules intercalate between these two residues, stabilizing the 280s loop and maintaining the enzyme in the T-state conformation. In the T-state, Arg 569 resides within a very buried environment, forming hydrogen bonds to the main-chain carbonyls of Asn 133, Pro 281 and Lys 608 and the side chain of Asn 133, with the guanadinium group ~7 Å from the catalytic site (Fig. 5*b*). In the absence of other ligands, phosphate does not bind to the catalytic site in the T-state[31,32].

On transition to the R-state, conformational changes at the catalytic site occur to convert it into a form capable of binding phosphate. This represents the essential feature of the allosteric transition; affinity of the catalytic site for phosphate is 15 times higher in the R-state than in the T-state[5,33]. The presence of a phosphate-binding site in the R-state is indicated by a strong isolated feature in the electron density map to which a sulphate of crystallization can be fitted (Fig. 5). The recognition site is created by adjustments in the torsion angles in the Arg 569 side chain, shifting the guanadinium group by 7 Å so that it swings into the catalytic site and displaces Asp 283. All other residues participating in the binding site are available in the T-state. Motion of Arg 569 is triggered by the displacement of Asn 133 and Pro 281, induced by motion of Ile 165, weakening interac-

TABLE 2 Residues involved in intersubunit van der Waals contacts, salt bridges and hydrogen bonds in the R-state and T-state structure

van der Waals interactions of interface residues <4.0 Å

Residue	T-state	R-state
N-terminal tail/α5–α6 loop' interface		
R10		L115', G116'
I13		L115'
Cap/α2' and N-terminal tail' interface		
N32		Q12', I13'
R33	R33'	L18', R33'
L35		I13'
H36	G65' I68', E839', F37'	I13', S14', V15'
F37	E839', H36', D61', V64'	L18', D61', V64'
V40	V64'	W67'
K41	I68'	I68'
N44		Q72'
Y51		R10', I13'
Cap/β7' interface		
T38	K191'	K191'
K41	R193'	R193', F196'
β6/β7' interface		
Y185	P194'	
Tower/β6' interface		
E177	L254'	
E178	N250'	
Tower/Tower' interface and tower/β5' interface		
Y262	F166', P281'	N264'
I263	Y280'	V259'
V266	F163', V278', Y262	L267', N270'
L267	L291'	V266'
R269	F163'	N270', R277'
N270	N270', R277'	V266', R269'

Salt bridges and hydrogen bond of interface residues <3.5 Å

T-state	R-state
N-terminal tail/β5–β6 loop' interface	
	R10:NH2–G116':O
Cap/α2' and N-terminal tail' interface	
H36:NE2–D838':OD2	N30:OD1–R33':NH1
N44:OD1–WAT–N72':OE1	R33:NH1–D61':OD1
	D42:OD2–Q72':NE2
	R43:NH1–SUL':O2
	R43:NH1–SUL':O4
	R43:NH2–I13':O
	R43:NH2–SUL':O3
Cap/β7' interface	
L39:O–K191':NZ	L39:O–K191':NZ
L39:O–R193':NH2	L39:O–R193':NH2
L40:O–R193':NH1	L40:O–R193':NH2
K41:NZ–E195':OE1	K41:NZ–E195':OE1
Tower/β6' interface	
E178:OE2–N250':ND2	
A179:N–N250':O	
D180:N–WAT–Y262':OH	
D181:OD1–WAT–R269':NH1	
Tower/tower' interface	
N270:ND2–N274':OD1	
R277:NH1–N274':OD1	

The surface area made inaccessible to solvent on formation of the subunit–subunit interface is 105 Å^2 larger for the T-state dimer than for the R-state dimer (2,322 Å^2 and 2,217 Å^2, respectively). Residues are identified by the one-letter code. Residues of the opposite subunit are designated by a prime. WAT, ordered water molecules in the T-state structure; SUL, sulphate. Assignment of secondary structural elements are for the T-state structure[30].

tions to Arg 569, and by the concerted motion of Asp 283. There is no visible electron density to account for residues 282–286 and it is assumed that these residues become mobile. Disorder of the 280s loop suggests that the gate to the catalytic site is removed, allowing oligosaccharides to bind and perturbing the inhibitor site, thus abolishing inhibition by nucleosides. Histidine 571 rotates about its C_α–C_β bond, following rupture of a hydrogen bond to Asp 283 made in the T-state, and forms a new hydrogen bond to Tyr 613.

The binding site for the phosphate of the substrate, formed from the side chains of basic residues Arg 569 and Lys 574, and the main-chain nitrogen of Gly 135, is situated adjacent to the phosphate of PLP. The phosphate of PLP and the sulphate are separated by 5 Å and a hydrogen bond is formed between the two anions. The contacts to the phosphate of the PLP are essentially the same in the T-state[26], namely hydrogen bonds to Lys 568, and the main-chain nitrogens of Thr 676 and Gly 677. The sulphate-binding site observed in the R-state is similar to

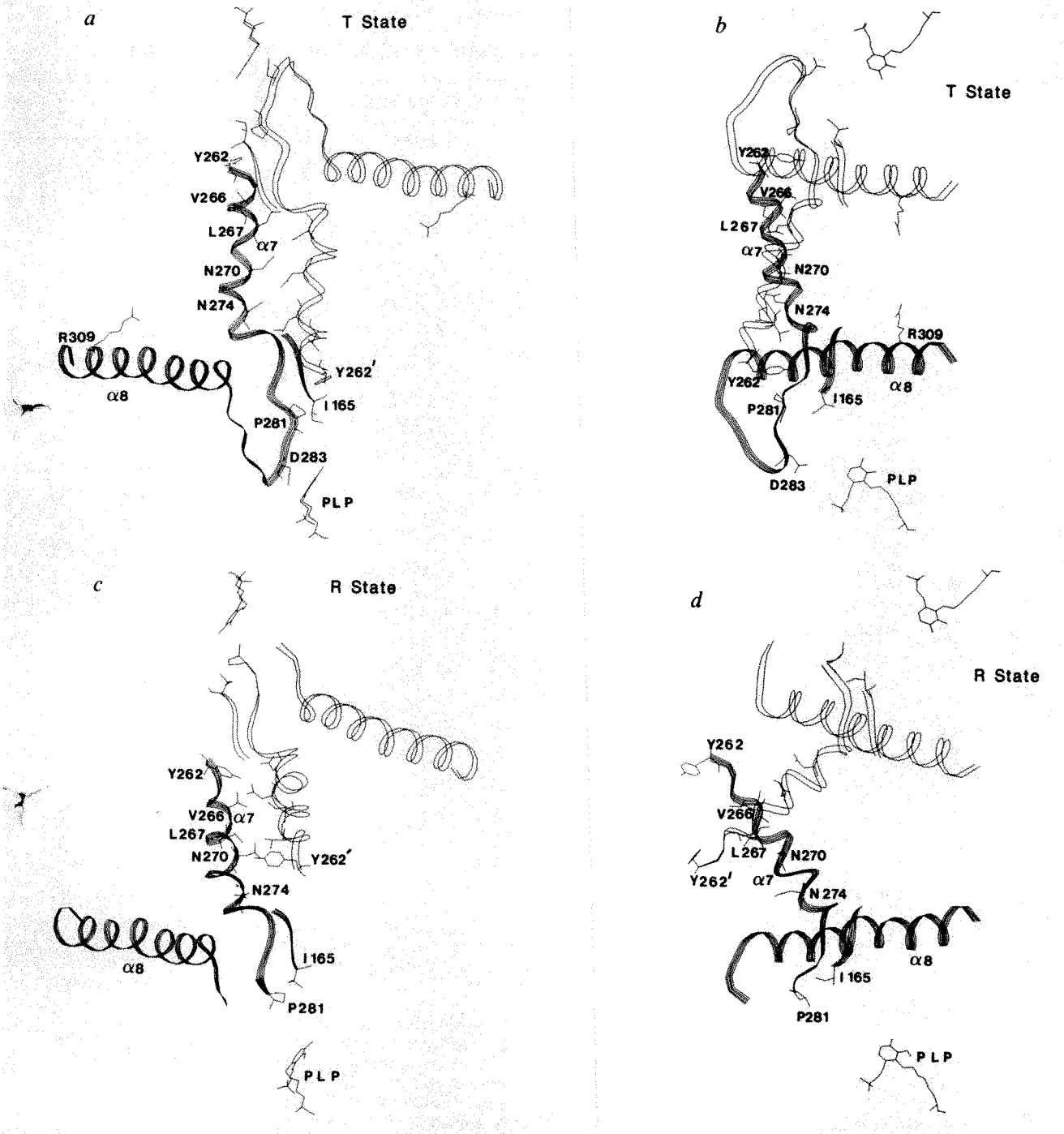

FIG. 4 Two orthogonal views showing the change in association of the symmetry-related tower helices (α7) on transition from the T-state (Fig. 4*a*,*b*) to R-state (Fig. 4*c*,*d*). The view in *a* and *c* is along the 2-fold axis of the T-state dimer. The view in *b* and *d* is perpendicular to the 2-fold axis and similar to that in Fig. 3. The ribbon traces residues 262 to 314 (T-state), residues 262 to 281 and 287 to 314 (R state), and residues 162 to 165 in both structures. These residues include the tower helix, the short parallel sheet between 276 to 279 and 162 to 164 and the α8 helix forming part of the rigid core, which superimpose in the lower subunits of both structures. Arginine 309, which contributes to the AMP phosphate recognition site, is included for reference in α8 of the T-state. The catalytic site is represented by PLP. Residues displayed are Ile 165, Tyr 262, Val 266, Leu 267, Asn 270, Asn 274, Pro 281, Asp 283 (T-state only), Arg 309 and Lys–PLP 680. The change in the cluster of Ile 165, Pro 281 and Tyr 262′ on T- to R-transition is apparent. For further details see text.

the phosphate-recognition site created in T-state GP*b* crystals when they bind the transition-state analogue, heptulose-2-phosphate[31,34,35]. Similar movements of Arg 569 are also observed. The glucosyl-binding site for the non-reducing end of a glycogen substrate is completely formed in the T-state structure and no change occurs on transition to the R-state.

The structural data presented here were anticipated by biochemical and spectroscopic studies. Arg 569 has been shown to be unreactive to phenyl-glyoxal reagents in the T-state, but reactive in the R-state and protected by glucose-1-P[36,37]. These observations are consistent with the burial of Arg 569 in the T-state, exposure in the R-state, and burial on the ligation of phosphate when binding glucose-1-P. The closeness of the 5′-phosphate of PLP to the substrate phosphate was indicated from the results of pyridoxal reconstitution studies of phosphorylase[38]. It has been shown by ^{31}P-NMR measurements that the ionization of PLP is sensitive to the activation of phosphorylase[39,40] On the transition from the T- to R-state, the phosphate of PLP converts from a mono-anion to a di-anion, and reverts to a mono-anion on ligation of the substrate phosphate. These changes can be understood by variations in the electrostatic environment on the T- to R-state conversion. In the T-state, Asp 283 is linked through two water molecules to the 5′-phosphate of PLP, favouring the mono-anionic form of the cofactor phosphate. In the R-state, Asp 283 is displaced by Arg 569, and the additional positive charge favours the di-anionic phosphate of the cofactor. Addition of phosphate or sulphate near to the 5′-phosphate promotes protonation.

Structural changes at the allosteric sites

Analogous to the tower–tower interface, structural interdependence of quaternary and tertiary changes is observed at the cap′/α2 interface, although the amplitude of both changes is smaller. Movement of the cap′ towards the α2 helix is facilitated

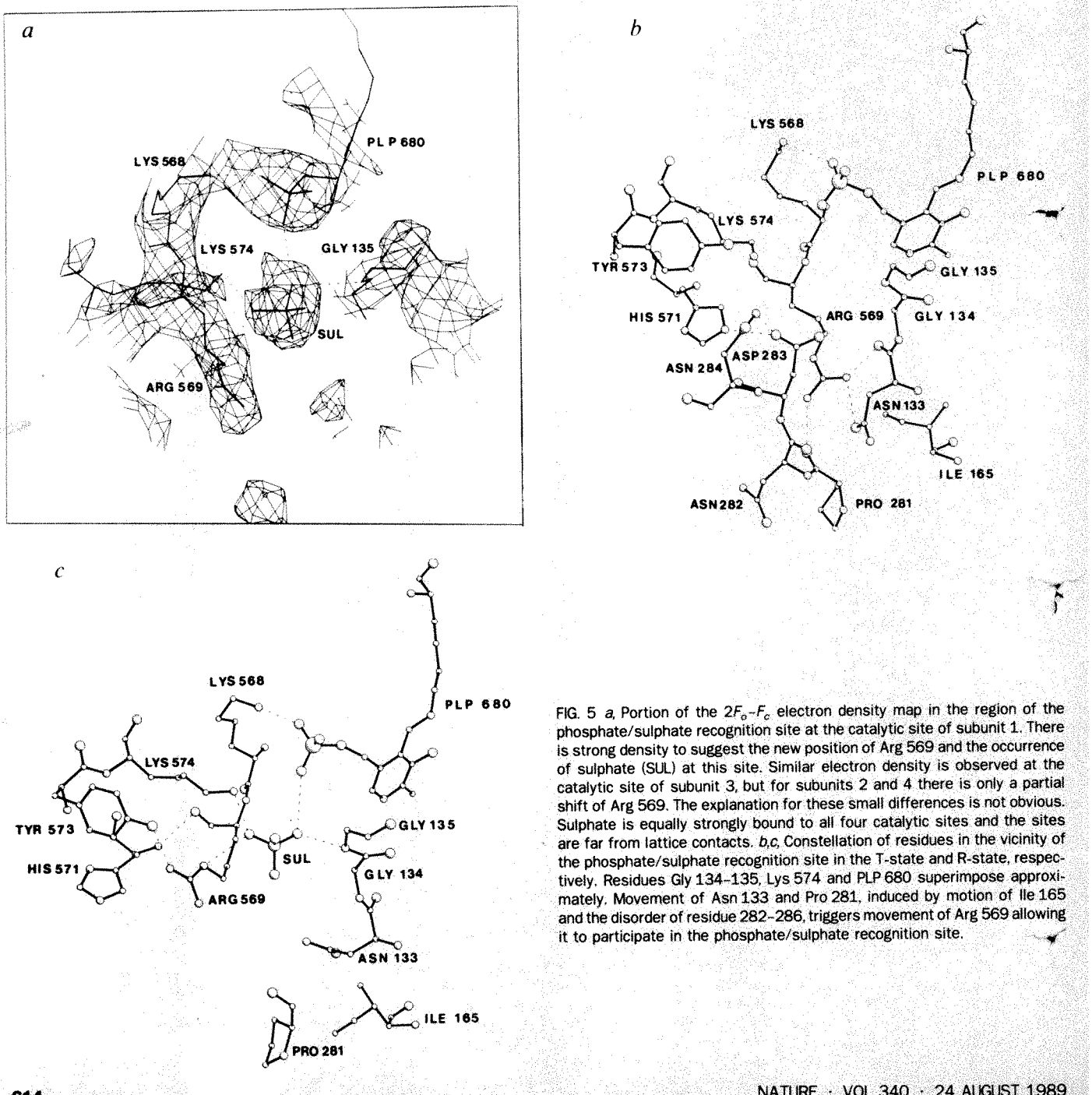

FIG. 5 *a*, Portion of the $2F_o$–F_c electron density map in the region of the phosphate/sulphate recognition site at the catalytic site of subunit 1. There is strong density to suggest the new position of Arg 569 and the occurrence of sulphate (SUL) at this site. Similar electron density is observed at the catalytic site of subunit 3, but for subunits 2 and 4 there is only a partial shift of Arg 569. The explanation for these small differences is not obvious. Sulphate is equally strongly bound to all four catalytic sites and the sites are far from lattice contacts. *b,c*, Constellation of residues in the vicinity of the phosphate/sulphate recognition site in the T-state and R-state, respectively. Residues Gly 134–135, Lys 574 and PLP 680 superimpose approximately. Movement of Asn 133 and Pro 281, induced by motion of Ile 165 and the disorder of residue 282–286, triggers movement of Arg 569 allowing it to participate in the phosphate/sulphate recognition site.

and stabilized by concerted motions of amino-acid side-chains of these structural elements, promoted by the binding of sulphate at the Ser-phosphate site.

In the R-state crystals, sulphate is bound close (2 Å) to the Ser-phosphate 14 of GP*a*, making a hydrogen bond to the side-chain of Ser 14 (Fig. 6). Residues 10–18, which are disordered in the T-state, become ordered. Rotations of the side-chains of Arg 43′ and Arg 69 allow the guanadinium groups to form hydrogen bonds with the sulphate. These interactions are only favourable following the closer association of the cap′ and α2 helix. Coupling of quaternary and tertiary changes is observed on the rotation of His 36′ about the C_α–C_β bond into a previously unoccupied cavity to prevent steric conflict with Ile 68 as the subunits rotate. Movement of His 36′ ruptures a salt bridge to Asp 838, and residues 838–841, which are ordered in the T-state, become disordered. The position previously adopted by residues 838–841 is occupied by Arg 16. Valine 15 packs into a pocket formed between His 36′ and Ile 68, and His 36′ forms hydrogen bonds to the main-chain oxygen of Asn 32′.

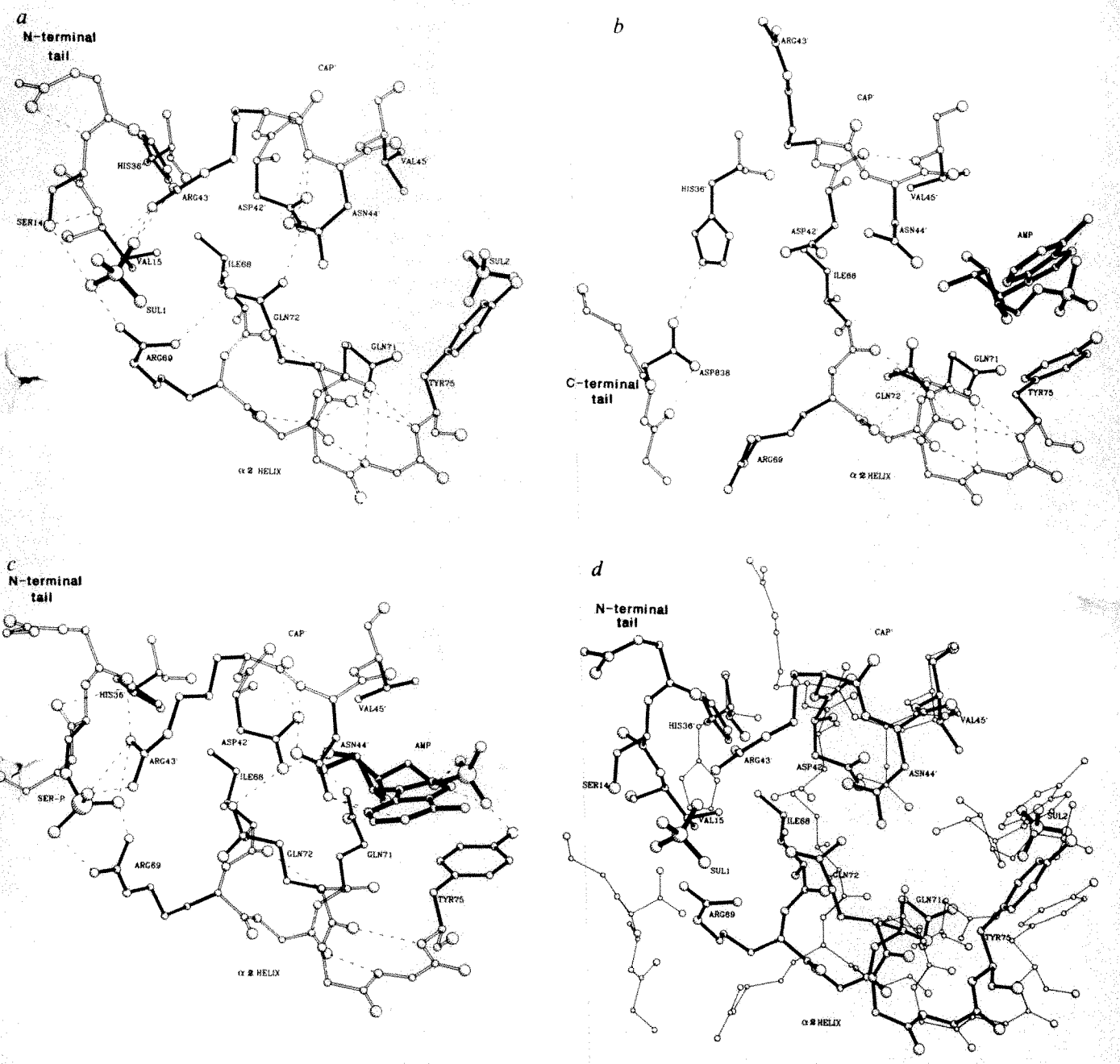

FIG. 6 Conformational changes at the cap′/α2 interface. *a*, GP*b* R-state; *b*, GP*b* T-state[30]; *c*, GP*a*-AMP complex T-state[48]; and *d*, GP*b* R-state (bold lines), GP*b* T-state (fine lines) superimposed. The cap′ and α2 helix are marked. The N-terminal tail of GP*b* R-state and GP*a* T-state, and C-terminal tail of GP*b* T-state are on the left. The subunit carrying the cap′ of T-state GP*b* and GP*a* have been superimposed onto the equivalent subunit of R-state GP*b* and the three structures have been drawn from the same orientation. The closer association of the cap′ to the α2 helix state is stabilized by salt bridges to Ser-phosphate in T-state GP*a*, or to sulphate (SUL1) in R-state GP*b* from Arg 43′ and Arg 69, and interactions between Asp 42′ and Gln 72. The N-terminal tail displaces the C-terminal tail, disrupting a salt bridge between His 36′ and Asp 838; subsequent rotation of His 36′ prevents steric conflict with Ile 68. The change in conformation of Asp 42′ Asn 44′, Gln 72 and Tyr 75 creates a site with high affinity for AMP in R-state GP*b* and T-state GP*a*. The sulphate (SUL2) bound in GP*b* R-state is close to the site for the phosphate of AMP and is shown on the right of *a* and *d*. The three arginines (Arg 242, Arg 309 and Arg 310) that stabilize this site have been omitted for clarity. (The GP*a* coordinates[15,48] were provided by S. R. Sprang, R. J. Fletterick and E. Goldsmith.)

The quaternary and tertiary structural changes that occur in the transition of GP*b* from the T- to R-state also result in an allosteric site with high affinity for AMP located 12 Å from the sulphate/Ser-phosphate site. In the T-state structure, His 36′ intercalates between Val 64 and Ile 68 of the α2 helix, inducing the helix to unwind by 1 Å towards its C-terminus. Rotation of His 36′ on transition to the R-state allows re-ordering and contraction of the helix, positioning Gln 72 so as to form a hydrogen bond to Asp 42′ of the cap′. Comparison with T-state GP*b* and GP*a* suggests that the side-chains of Asp 42′, Asn 44′ and Gln 72 are correctly positioned to hydrogen bond to AMP, although this has to be confirmed by direct binding studies.

Both AMP and Ser-phosphate 14, or sulphate, stabilize the R-state by interacting with residues from both subunits that are only available in the R-state conformation. Communication between Ser-phosphate 14 and the allosteric effector site is mediated through a closer association of the cap′ and α2 helix, and re-ordering of the α2 helix. Thus, although the enzyme does not possess a phosphorylated serine, in the presence of sulphate the protein conformation at this interface is almost identical to that of the glucose-inhibited T-state GP*a*[15] (Fig. 6).

Discussion

The allosteric transition of glycogen phosphorylase can be simply described. The transition from the T-state to the R-state involves small changes in tertiary structure at the ligand-binding sites and the subunit interface regions, and little change in the remainder of the subunit. These are coupled to large changes in quaternary structure that involve rotation of the two subunits with respect to one another. The change in quaternary structure directly affects the allosteric effector site and the Ser-phosphate site, which are located at the cap′/α2 interface, and transmits a signal to the catalytic site and nucleoside-inhibitor site through conformational changes of the two tower helices. Movement of the tower helix indirectly leads to a replacement of an aspartate by an arginine residue at the catalytic site, and the opening of a tunnel to allow access for substrate. The crystallographic studies show tertiary structural changes in response to ligand binding[41]. Each of these changes includes a movement of arginine residues to create a phosphate- (or sulphate-) recognition site. The correct position of arginine side chains is coupled to the presence of an anion at these sites. At the catalytic site, the movement of Arg 569 contributes to the stabilization of the close proximity of the substrate anion to the cofactor 5′-phosphate, which is an essential part of the enzymic mechanism. At the Ser-phosphate site, two arginines, one from each of the subunits, move to create the phosphate site, and the presence of the di-anion leads to a re-ordering of the basic N-terminal tail. The major feature in creating the high affinity allosteric site is the movement of the cap′/α2 interface, but the phosphate of AMP, which is essential for activation, also induces movement of an arginine, (Arg 309) to create with Arg 310 a site with high affinity for phosphate.

The alternate packing adopted by the tower helices provides a particularly suitable method by which to couple tertiary and quaternary conformations. A rotation of the two subunits in the allosteric transition alters the relative disposition of the two helices. To achieve more favourable helix packing-geometry a further tertiary structural change occurs that affects the relative disposition of the helices. Because the geometry of one helix is constrained by that of the symmetry related helix, molecular symmetry is preserved and a concerted conformational change of both subunits occurs. A strict coupling of quaternary to tertiary structure is required so that heterotropic allosteric effectors bound at the cap′/α2 interface, 35 Å from the tower helix interface, can transmit a conformational change to the catalytic site. Thus homotropic and heterotropic mechanisms involve the same gross structural changes and are mutually interdependent. Phosphorylase was one of the first enzymes whose kinetic properties were described by a two-state interpretation of the concerted allosteric model of Monod, Wyman and Changeux[3,42]. Our account of the structural transition agrees closely with that envisaged in this model. Two quaternary conformations have been characterized by the alternative packing of the tower helices.

The change in helix packing of the tower helices is quite different from the much smaller helix-shear interface movements recognized for the conformational change observed in citrate synthase[43] and different structures of insulin[44]. The transmission of allosteric effects through changes in helix–helix packing as seen in phosphorylase, or through helix-shear movements, may provide a basis for understanding receptor-mediated transmembrane signalling. □

Received 18 May; accepted 10 July 1989.

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ACKNOWLEDGEMENTS. We thank Drs K. R. Acharya, D. I. Stuart and P. J. McLaughlin for help with data collection and the refined coordinates of T-state GP*b*, G. L. Taylor for computational facilities, P. D. Jeffrey for discussion, and the staff at the Synchrotron Radiation Source, Daresbury for excellent facilities. This work was funded by the MRC and SERC. L.N.J. is a member of the Oxford Centre for Molecular Sciences.

A submillisecond pulsar and the equation of state of dense matter

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If the submillisecond pulsar in the remnant of supernova 1987A really is rotating stably with a period P_{smp} of 0.508 ms (ref. 1), its existence can be used to rule out nearly all 'realistic' equations of state for dense nuclear matter. (An alternative hypothesis, that the pulsar is vibrating rather than rotating[2], yields no such constraints.) We present here a simple equation of state that yields, in the non-rotating case, maximally compact models of neutron stars, and argue that stars constructed in this way will also be stable when rotating with periods <0.5 ms. Additional constraints found by applying the same equation of state to the 'slowly' rotating pulsar PSR1913+16, whose mass is accurately known, leaves only a small range of acceptable parameters for neutron star models based on equations of state that obey the causality requirement that their sound speeds are less than the speed of light.

Rapid rotation may induce neutron-star instability (see ref. 3 and refs therein). The discovery of the millisecond pulsar PSR 1937+214 (which had the highest known rotational frequency, $\Omega = 4.03 \times 10^3\ s^{-1}$, before the announcement in ref. 1) implies some mild constraints on the parameters of possible neutron-star models[3]. The existence of a submillisecond pulsar with $\Omega_{smp} = 1.24 \times 10^4\ s^{-1}$, however, is expected to imply more stringent constraints.

An object of mass $M \approx M_\odot$ and radius $R < 10$ km that is rotating with a frequency of $\sim\Omega_{smp}$ requires a relativistic treatment of both rotation and gravity. Such a calculation has been recently done for a uniformly rotating neutron star[4]. For a rather broad set of dense-matter equations of state the following sequence of neutron-star instabilities is induced by an increasing Ω. First, the gravitational-radiation non-axisymmetric instabilities appear for the $m = 4$ and then for the $m = 3$ perturbations. Second, for a slightly higher $\Omega = \Omega_K$, the Kepler frequency is reached at the equator and mass shedding begins. Because no uniformly rotating star can reach $\Omega > \Omega_K$, the Kepler frequency sets a very rigid upper limit on the possible uniform rotation in neutron-star models. None of the equations of state studied[4] can give $\Omega_K > \Omega_{smp}$ for the configuration with the 'standard' baryon mass, $M_B = 1.4 M_\odot$. Actually, the condition for the stability in the rotating neutron-star model is even stricter—instead of Ω_K, we should use a slightly lower value Ω_{lim}, corresponding to the gravitational radiation $m = 3$, 4 instabilities. In practice, however, we can approximate $\Omega_{lim} \approx \Omega_K$ (ref. 4). This approximation is particularly good for equations of state yielding models of very compact neutron stars, which are the only promising candidates for the submillisecond pulsar model.

The value of Ω_K increases with increasing M_B and reaches its maximum $\Omega_{K,max}$ for the configuration with a maximum allowable mass. $\Omega_{K,max} > \Omega_{smp}$ can only be obtained for one equation of state[5] considered in ref. 4 but the maximum mass of a non-rotating neutron star $M_{max}(\Omega = 0)$ obtained using this equation of state is smaller than $M_{PSR1913+16} = 1.45\ M_\odot$ (ref. 6) (PSR1913+16 can be well approximated as a non-rotating neutron star because $P_{PSR1913+16} = 59\ ms \gg 2\pi/\Omega_K$.) For the equations of state of Arponen[7] and Friedman and Pandharipande[8] $\Omega_{K,max} \approx \Omega_{smp}$, within <1%, and thus we should expect that $\Omega_{lim,max} < \Omega_{smp}$. We conclude that, for uniform rotation, the constraint $\Omega_{K,max} > \Omega_{smp}$ combined with $M_{max}(\Omega = 0) > M_{PSR1913+16}$ rules out all five 'realistic' equations of state of dense matter studied in detail in ref. 4. (The assumption of uniform rotation is based on our belief that the dissipation of differential rotation in a newly born, hot neutron star occurs on a timescale much shorter than two years.)

To obtain a graphical representation of the $\Omega_K > \Omega_{smp}$ constraint in the M–R plane for the non-rotating neutron-star models, we introduce an analytical formula for $\Omega_{K,max}$ that reproduces the numerical results[4] very well (<5% error)

$$\Omega_{K,max} = 7.7 \times 10^3 \left(\frac{M_{max}}{M_\odot}\right)^{1/2} (R_{M_{max}})^{-3/2}\ s^{-1} \qquad (1)$$

where M_{max} and $R_{M_{max}}$ are the maximum allowable mass and the corresponding stellar radius (in units of 10 km) for the non-rotating configurations. Generally, equation (1) works extremely well for sufficiently compact configurations—which is the case for the maximum-allowable-mass configurations. Equation (1) is not based on relativistic considerations so its successful application to neutron-star pulsars is surprising.

Using equation (1), we write a necessary condition for a given dense-matter equation of state to give uniformly rotating neutron-star models that are consistent with a given observed pulsar period P (in ms),

$$\frac{M_{max}}{M_\odot} > 0.67 (P)^{-2} (R_{M_{max}})^3 \qquad (2)$$

where the values of M_{max} and $R_{M\,max}$ (in units of 10 km) refer to the non-rotating neutron-star models with the maximum allowable mass. Condition (2) has the same functional form as the stability conditions for the uniform rotation discussed in ref. 3. Equation (2) has, however, the practical advantage of being fitted to the results of numerical general-relativity calculations[4] for uniformly rotating neutron-star models from a broad range of dense-matter equations of state. There is a significant

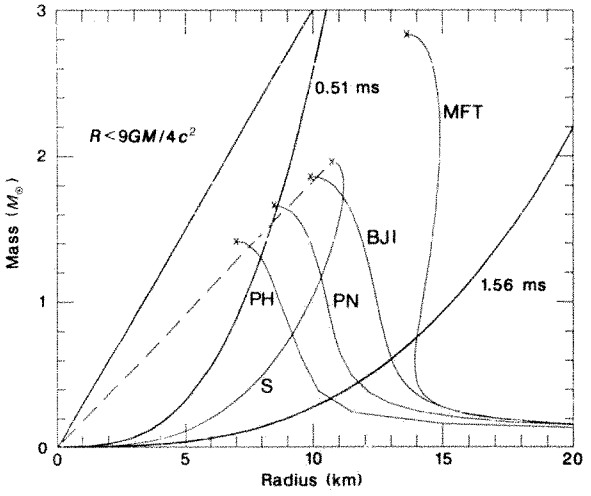

FIG. 1 Allowed regions in the M–R plane for the non-rotating neutron-star models. Configurations with the maximum allowable mass are denoted by crosses. The limiting curves for the extreme configurations have been obtained using equation (2). Equations of state for which the extreme configurations lie to the right of the solid curve marked 1.56 ms are excluded by the existence of the millisecond pulsar PSR1937+214, whereas those that yield the extreme configurations lying to the right of 0.51 ms curve are excluded by the pulsar announced in ref. 1. Configurations with $R < 9GM/4c^2$ are prohibited by general relativity (see, for example, ref. 18). The $M(R)$ curves for various equations of state are: MFT–mean-field theory, model 0.17 of Haensel *et al.*[19]; BJI–model I of Bethe and Johnson[20]; PN–Pandharipande equation of state for neutron matter[9]; PH–Pandharipande equation of state for the hyperonic matter[10]; S–strange-quark stars for the simplest MIT bag model with $B = B_0 = 60\ MeV\ fm^{-3}$. The maxima of the strange-star $M(R)$ curves corresponding to $B > B_0$ lie on the dashed line connecting the maximum of the S curve with the point $M = 0$, $R = 0$.

difference in the numerical coefficients: our value is 0.67, compared with at least 0.88 from Table 1 of ref. 3.

In Fig. 1 we show M against R for non-rotating star models for several dense-matter equations of state. Although all 'realistic' equations of state easily satisfy condition (2) for $P = P_{\mathrm{PSR1937+214}}$, only the 'soft' matter ones can, marginally, fulfil condition (2) for $P = 0.51$ ms. The PN model[9] fulfils this condition only for the configurations in which the mass is very close to $M_{\max}$, and the PH equation of state[10] fails to satisfy the constraint $M_{\max} > M_{\mathrm{PSR1913+16}}$. Therefore, to satisfy the condition (2) and the constraint for $M_{\max}$ we are forced to choose the equation of state very carefully; it must be somewhat softer than the PN one, but stiffer than the PH equation of state. Even then condition (2) and the constraint $M_{\max} > M_{\mathrm{PSR1913+16}}$ allow for the existence of a very narrow range (with masses very close to the maximum allowable mass) of neutron-star models that are stable at $P = P_{\mathrm{smp}}$.

If strange quark matter is the absolute ground state of matter at zero pressure[11], then we can consider the possibility of strange stars[11-14]. The macroscopic parameters of strange stars, in particular their M-R relationship, are determined primarily by the MIT (Massachusetts Institute of Technology) bag-model constant B. We consider the simplest model of free massless quarks[11]. Figure 1 shows an example of the M-R curve of the strange stars, for $B = 60\ \mathrm{MeV\,fm^{-3}}$. The parameters of the maximum-allowable-mass configurations with scale with the value of B according to[11,12]

$$M_{\max} = 1.96\left(\frac{B_0}{B}\right)^{1/2} M_\odot$$
$$R_{M_{\max}} = 10.7\left(\frac{B_0}{B}\right)^{1/2} \mathrm{km} \qquad (3)$$

where $B_0 = 60\ \mathrm{MeV\,fm^{-3}}$, so that the extreme configurations lie on the straight line in the M-R plane

$$\frac{2GM}{Rc^2} = 0.54 \qquad (4)$$

At first glance Fig. 1 seems to show that there exists a large area corresponding to models of strange-stars that can rotate at $\Omega > \Omega_{\mathrm{smp}}$. The condition for the self-bound state of strange matter to be energetically preferred over the normal, nucleonic one, is, however, satisfied only for $B < 1.5B_0$ (ref. 14) and this excludes the possibility of $\Omega = \Omega_{\mathrm{smp}}$ for strange stars. The inclusion, within the MIT bag model, of the finite mass of the strange quark and of the quantum chromodynamic interaction between quarks[12,13] does not change this conclusion. We note that strange-star models are also ruled out for some of the radio pulsars, namely those exhibiting glitches[15]. (The mass of the solid crust of strange stars is too small ($<10^{-5}M_\odot$ (ref. 13)) to account for the observed discontinuities in the pulsar timing.)

A question of practical importance arises: what is the equation of state that is optimum from the point of view of maximum stability of the uniformly rotating neutron star and that is consistent with the general physical requirements, as well as with the constraint $M_{\max}(\Omega = 0) > M_{\mathrm{PSR\,1913+16}}$? To obtain a massive object of maximum mean density, our equation of state should correspond to matter of maximum stiffness (velocity of sound = c) that is also very dense, even at pressure $P = 0$. This corresponds to

$$P = (\rho - \rho_0)c^2 \qquad (5)$$

where ρc^2 is the energy density of matter. Our equation of state contains only one free parameter ρ_0, which will determine the value of $M_{\max}$. Matter described by equation (5) has a superdense self-bound state at $P = 0$, which is reminiscent of the 'abnormal' state of matter, discussed in the mid-1970s[16].

The M-R curves for the self-bound, causality-limit equation of state, equation (5), are shown in Fig. 2. For $\rho_0 = 0.84 \times 10^{15}\ \mathrm{cm^{-3}}$ we get $\Omega_{\mathrm{K,max}} = \Omega_{\mathrm{smp}}$ at $M_{\max}(\Omega = 0) = 2.31\ M_\odot$. For a sufficiently high ρ_0, all configurations are stable at $\Omega = \Omega_{\mathrm{smp}}$. Non-rotating configurations of maximum mass depend in a very simple way on the parameter ρ_0,

$$M_{\max}(\rho_0') = \left(\frac{\rho_0}{\rho_0'}\right)^{1/2} M_{\max}(\rho_0)$$
$$R(\rho_0') = \left(\frac{\rho_0}{\rho_0'}\right)^{1/2} R(\rho_0) \qquad (6)$$

so that the maxima of the $M(R)$ curves corresponding to different ρ_0 lie on the straight line

$$\frac{2GM}{Rc^2} = 0.71 \qquad (7)$$

The straight line, equation (7), is very similar to that corresponding to an absolute upper bound for the surface redshift of neutron stars[17]. Thus, the condition of causality (velocity of sound $\leq c$) leaves us with a rather large area of compact configurations that can rotate uniformly with $\Omega > \Omega_{\mathrm{smp}}$ but, if we impose the additional constraint $M_{\max}(\Omega = 0) > M_{\mathrm{PSR1913+16}}$, then the permitted area for the maximum-mass configurations reduces to a rather small 'triangle'.

Having considered various possibilities for constructing very compact neutron-star models, we can conclude that the possible existence of a submillisecond pulsar with $P_{\mathrm{smp}} = 0.5$ ms could be reconciled with a theory of neutron stars that is based on general relativity and a causal equation of state. We are then forced, however, to accept a rather narrow range of the models of dense matter that yield neutron stars having the maximum mean density, and we must reject nearly all existing 'realistic' equations of state. This statement can be significantly weakened if we consider the possibility that the 'abnormal' state of matter, which leads to very compact neutron stars, is separated from the 'normal' one, described by the standard equations of state, by a large energy barrier. Then, depending on the scenario of a neutron-star's formation, it could be in a 'normal' or an 'abnormal' state. The observation of a submillisecond pulsar may be relevant only for the 'abnormal' neutron stars, just because this pulsar is an 'abnormal' star and the condition

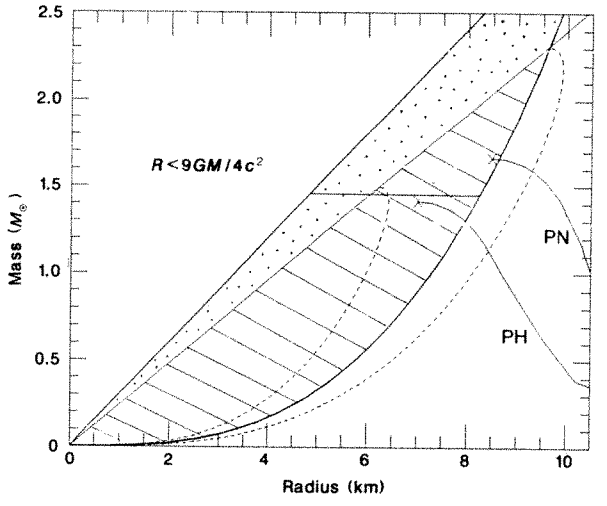

FIG. 2 Heavy lines are the boundaries of the region of extreme non-rotating neutron-star configurations allowed by general relativity ($R > 9GM/4c^2$) and by the characteristics of the pulsar announced in ref. 1 ($\Omega_{\mathrm{K,max}} > \Omega_{\mathrm{smp}}$). Dashed lines: the $M(R)$ curves for the self-bound, causality-limit equation of state, equation (5), for $\rho_0 = 8.4 \times 10^{14}\ \mathrm{g\,cm^{-3}}$ (right curve) and for $\rho_0 = 2.0 \times 10^{15}\ \mathrm{g\,cm^{-3}}$ (left curve). The hatched area corresponds to the causal equations of state. If the causality requirement is relaxed, the allowed region is augmented by the dotted sector. The horizontal line corresponds to $M = M_{\mathrm{PSR1913+16}}$.

$M_{max} > M_{PSR1913+16}$ could be only relevant for 'normal' neutron stars, because this binary radio pulsar happens to be a 'normal' neutron star. □

Received 13 March; accepted 27 June 1989.

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Oxygen ordering, phase separation and the 60-K and 90-K plateaus in $YBa_2Cu_3O_x$

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A PLOT of the superconducting transition temperature (T_c) against oxygen content (x) for $YBa_2Cu_3O_x$ exhibits two 'plateaus' when oxygen is removed from the material at low temperatures. T_c remains nearly constant at ~60 K for $x = 6.6$–6.7 and nearly constant at ~90 K for $x = 6.8$–7.0. It is now common to assume that there are two distinct superconducting phases in $YBa_2Cu_3O_x$, the '60-K' and '90-K' phases, and that the two plateaus correspond to single-phase regions of the respective phases[1,2]. $YBa_2Cu_3O_x$ samples prepared at low temperatures contain a variety of ordered oxygen superstructures[3–5]. Several theoretical studies have tried to predict the phase equilibria between these ordered structures[6,7] and to explain how oxygen ordering leads to the T_c plateaus[8,9], but no clear understanding of the interrelationships amongst these factors has yet emerged. This is partly because the techniques used previously to prepare the low-temperature samples do not control or monitor all of the key processing variables, particularly the oxygen partial pressure. Here we report on the ordered oxygen structures and superconducting properties of $YBa_2Cu_3O_x$ samples prepared in precisely controlled oxygen environments using a solid-state ionic technique. We find no evidence for phase separation between structures that have widely different oxygen content, but we do see electron diffraction evidence for phase separation between distinct phases that differ only slightly in oxygen content, and these regions of phase separation coincide with the T_c plateaus. These results show that the commonly held view that the two plateaus correspond to single-phase regions of respective 60-K and 90-K phases is incorrect: the changes in superconducting properties with oxygen content in $YBa_2Cu_3O_x$ cannot be explained on this basis.

The solid-state ionic cell used to prepare the samples is described elsewhere[10–13]. $YBa_2Cu_3O_{7.00\pm0.02}$ starting material (oxygen stoichiometry measured by iodine titration) was heated to 500 °C, and then oxygen was titrated out until the desired oxygen content was reached, a process that took typically 2–3 days. The sample remained at 500 °C for a further day and was then slowly cooled to 25 °C by stepping down the furnace temperature over a 28-h period. Thus the ordered structures observed here were those that were frozen in as the temperature was slowly reduced to room temperature. These structures do not necessarily correspond to the ordered structures present at 500 °C.

Figure 1 summarizes the T_cs and ordered oxygen structures (denoted by their superlattice wavevectors) as a function of oxygen content. All of the electron diffraction patterns taken from different crystals in the same sample were essentially identical, except for the $x = 6.65$ and $x = 6.90$ samples (see below). Figure 2 is a series of line traces through electron diffraction peaks running along the a^* direction in selected samples, all taken from [001]-zone-axis patterns. The largest peaks in these traces are fundamental reflections, whereas the smaller peaks are superlattice reflections arising from oxygen ordering in the basal plane of $YBa_2Cu_3O_x$. No superlattice reflections were observed in tetragonal material. In particular, no $\langle\frac{1}{4}\frac{1}{4}0\rangle$ superlattice wavevector was found[14].

The ordered structure characterized by a $\langle\frac{1}{2}00\rangle$ superlattice wavevector, which is often called the 'Ortho II' phase, was seen in all crystals for $x = 6.28$–6.61 and in some crystals for $x = 6.65$. At $x = 6.28$, this superstructure was barely discernible in 10-nm orthorhombic microdomains in a 'tweed' microstructure. At higher oxygen content, it was clearly present in a macroscopically twinned microstructure. [010]-zone-axis patterns from Ortho II crystals in the $x = 6.50$–6.65 samples typically showed sharp superlattice reflections (Fig. 3), indicating that there was long-range ordering of this superlattice along the c^* direction. Dark-field images using superlattice reflections showed that this ordering occurred throughout crystals with intense $\langle\frac{1}{2}00\rangle$ reflections (Fig. 4), and not just as the isolated microdomains reported previously[15]. The electrical properties of the Ortho II phase varied considerably with oxygen content, ranging from an antiferromagnetic insulator at $x = 6.28$ to a 60-K superconductor at $x = 6.61$. It is clearly a misnomer to refer to Ortho II as just the '60-K phase'.

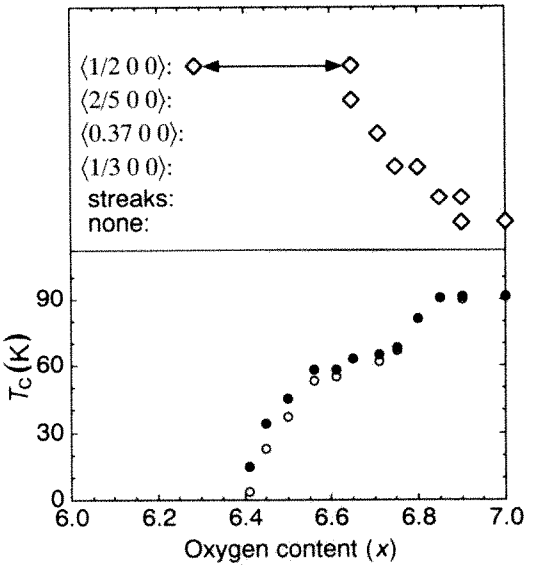

FIG. 1 Superconducting transition temperature T_c and ordered oxygen structures (denoted by their superlattice wavevectors) plotted against oxygen content. The only samples with more than one type of oxygen ordering are the $x = 6.65$ and $x = 6.90$ samples. ●, Diamagnetic onset; ○, Zero resistance.

Both the Ortho II phase and an ordered structure with $\langle\frac{2}{5}\,0\,0\rangle$ wavevector were found in the $x=6.65$ sample (Fig. 2). A progression of ordered structures occurred for $x=6.71$–6.90, and the Ortho II phase was no longer present (Figs 1 and 2). These structures, unlike the Ortho II phase, did not exhibit long-range correlation along the c^* direction. At $x=6.90$, electron diffraction patterns for some crystals displayed very diffuse streaks along a^* (as in $x=6.85$ sample), whereas diffraction patterns for other crystals contained no evidence for further oxygen ordering (as in the $x=7.00$ starting material). All of the ordered structures are consistent with the removal of entire chains of oxygen along the b axis of $YBa_2Cu_3O_x$. This type of ordering minimizes the number of threefold-coordinated copper in the basal plane. Moreover, it allows for the creation of Cu^+ ions even in samples with $x>6.5$.

These results show that the two T_c plateaus do not correspond to single-phase regions of respective 60-K and 90-K phases. If they did, only one type of ordering would be seen across each plateau and a mixture of these two orderings would occur between the plateaus. Figure 1 shows that this not the case. The 60-K plateau has the $\langle\frac{1}{2}\,0\,0\rangle$ superlattice at $x=6.61$, a mixture of the $\langle\frac{1}{2}\,0\,0\rangle$ and $\langle\frac{2}{5}\,0\,0\rangle$ superlattices at $x=6.65$, and the $\langle 0.37\,0\,0\rangle$ superlattice at $x=6.71$. The 90-K plateau has diffuse streaks along a^* at $x=6.85$ and no signs of further ordering at $x=7.00$. Between the two plateaus, there is a progression of ordered structures, not a mixture of crystals with the $\langle\frac{1}{2}\,0\,0\rangle$ superlattice and crystals with no signs of additional ordering. Thus, the changes in superconducting properties with oxygen content in $YBa_2Cu_3O_x$ cannot be explained simply by two separate and distinct superconducting phases corresponding to the 60-K and 90-K plateaus.

In fact, these results show that the ranges of x for which T_c is changing rapidly correspond to single-phase regions. (The sequence of ordered structures for $x=6.7$–6.9 behaves like a single nonstoichiometric phase.) The T_c plateaus, however, may correspond to two-phase regions. This behaviour is consistent with that expected when the Gibbs phase rule is applied to a pseudobinary system; intrinsic properties can vary in single-phase regions, but they are fixed in the coexisting phases by virtue of the phase rule[16]. Finding more than one type of diffraction pattern in the $x=6.65$ and $x=6.90$ samples is consistent with the idea that phase separation between ordered structures with only small differences in oxygen content may be occurring at these average stoichiometries. Moreover, a recent coulometric-titration study[17] supports the possibility of a low-temperature miscibility gap centred at $x=6.65$. Several caveats need to be mentioned, however, regarding this speculation that the T_c plateaus arise from phase separation. First, at equilibrium in off-stoichiometry samples, there should be phase separation into perfectly ordered phases at low temperatures, but complete phase separation may be difficult to achieve because of limited

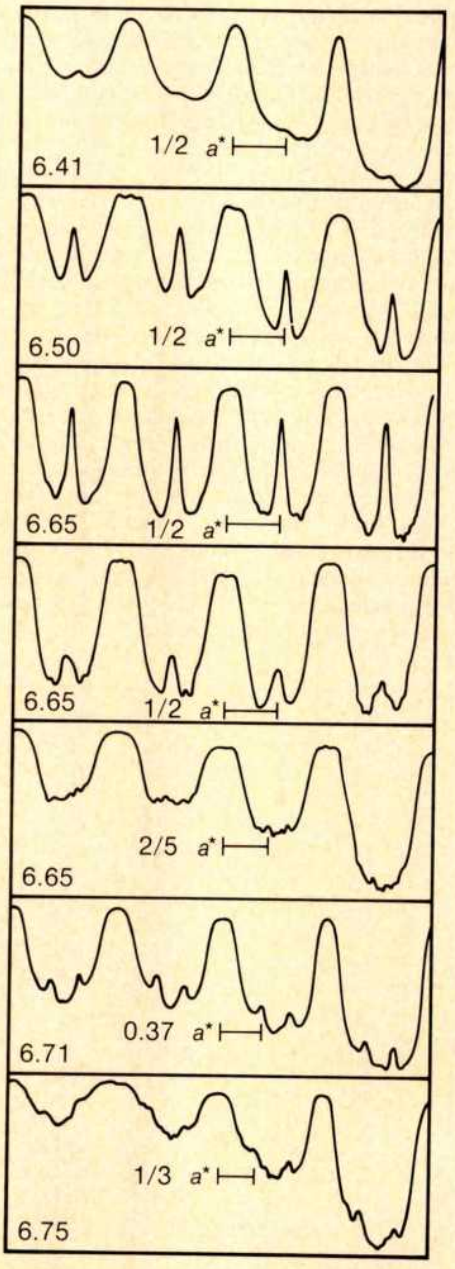

FIG. 2 Electron diffraction pattern line traces along the a^* direction showing fundamental and superlattice reflections in selected samples. The average oxygen content and superlattice wavevector for each sample are indicated.

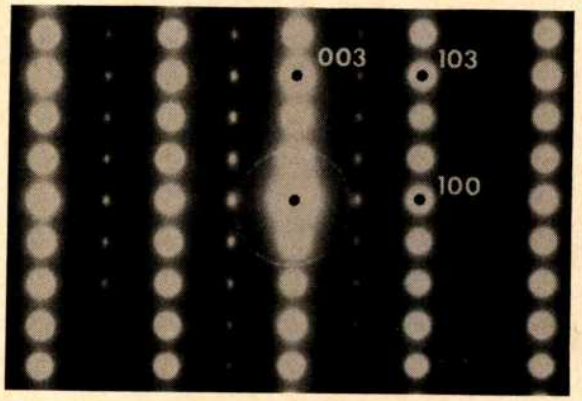

FIG. 3 Typical [010]-zone-axis pattern from the Ortho II phase, indicating that the $\langle\frac{1}{2}\,0\,0\rangle$ superlattice has long-range correlation in the c^* direction.

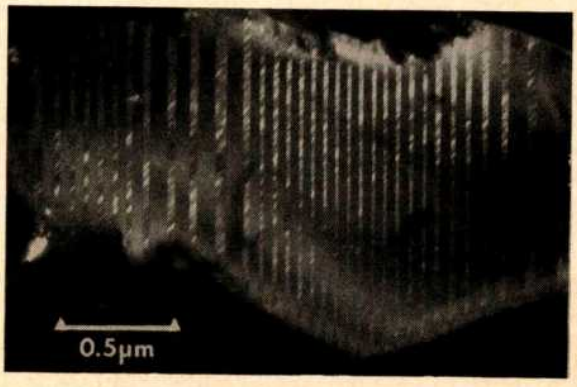

FIG. 4 Dark-field image of the Ortho II phase using $\langle\frac{3}{5}\,0\,0\rangle$ superlattice reflection. Oxygen ordering occurs throughout the crystal.

oxygen mobility at low temperatures. Thus, the plateaus may be 'smeared out' in nonequilibrated samples and even absent in samples rapidly quenched from high temperatures. Second, the apparent phase separation at $x = 6.90$ could also be caused by nonuniform oxygen uptake during the slow cooling because there is an appreciable oxygen pressure in the apparatus at this stoichiometry. (This caution does not apply to the $x = 6.65$ sample.) Third, because electron diffraction examines an exceedingly small portion of each sample, it would be useful to see if complementary measurements of the structure and properties of the bulk material also provide evidence for phase separation at the same stoichiometries. We note, however, that phase separation between phases with only small differences in oxygen content may be difficult to detect using bulk techniques. Alternatively, the ordered structures and T_c plateaus could be determined in $YBa_2Cu_3O_x$ derivatives to see if similar correlations are found. □

Received 19 June; accepted 1 August 1989.

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ACKNOWLEDGEMENTS. We thank R. Sinclair for the use of his electron microscope, J. H. Turner for help with image processing of the electron diffraction patterns, and P. Grant, F. Herman and J. Torrance for their comments.

Efficient photovoltaic devices for InP semiconductor/liquid junctions

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AN alternative to conventional solid-state photovoltaic devices is the semiconductor/liquid junction. Liquid-junction cells not only offer the possibility of integrating energy conversion and storage functions[1], but also may exhibit electrical properties that are fundamentally different from those in solid-state systems[2]. We have investigated the photovoltaic behaviour of n-InP/metal and n-InP/liquid junctions. We have found that the electrical properties of these semiconductor/liquid junctions are superior to those of n-InP/metal (Schottky barrier) systems, and that the current–voltage characteristics are a strong function of the electrochemical potential of the liquid phase. Liquid contacts thus provide a possibility for the construction of more efficient photovoltaic devices than those available at present from Schottky barriers.

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The n-InP samples were 300-μm-thick, 2×10^{16} cm^{-3} Si-doped crystals oriented along the (100) plane obtained from Crystacomm Inc., Mountain View, California. After mounting[3] and etching[4], the samples were immersed in the appropriate electrolyte solution and current–voltage (I–V) data were collected. The redox potential $E(A^+/A)$ of the $LiClO_4$–CH_3OH electrolyte was varied by use of several different pairs of oxidized (A^+) and reduced (A) ions. Because of their different electron affinities and solvation properties, addition of these redox pairs (A^+/A) to the liquid produced changes in the electrochemical potential (that is, the Fermi level) of the solution phase. On the electrochemical potential scale, more positive redox potentials indicate that the Fermi level of the solution is further away from the vacuum level. Thus, the difference in energy between the conduction band of an n-type semiconductor and the redox potential of the solution (that is, the barrier height) should be larger for more positive solution redox potentials. The molecules used included: cobaltocene$^{+/0}$ (with a standard electrochemical potential $E^{0\prime} = -0.9$ V relative to the reference level of a standard calomel electrode (SCE) ($CoCp_2$); decamethylferrocene$^{+/0}$ ($E^{0\prime} = -0.08$ V relative to SCE) ($Me_{10}Fc$); 1,1′-dimethylferrocene ($E^{0\prime} = +0.26$ relative to SCE) (Me_2Fc).

Figure 1 displays the dark I–V properties of n-InP/Au Schottky contacts and n-InP/0.20M Me_2Fc–0.10M Me_2Fc^+–1.0M $LiClO_4$–CH_3OH junctions. In accord with previous reports, we observed that the Schottky contacts exhibited ohmic behaviour at moderate current densities and were poor rectifying contacts with high reverse-saturation-current (J_0) values[5–9]. By contrast, the dark I–V properties of the dimethylferrocene–CH_3OH liquid contact had excellent rectifying characteristics and low J_0 values ($J_0 = 10^{-9}$ A cm^{-2}).

This difference in rectification implies that more efficient photoelectrochemical behaviour should be obtainable from the n-InP/liquid interface. Figure 2 displays the potentiostatic photoelectrode I–V properties of the n-InP/0.20M Me_2Fc–0.5mM Me_2Fc^+–1.0M $LiClO_4$–CH_3OH junction under simulated Air Mass 2 conditions (62 mW cm^{-2}) and under lower-level illumination. At Air Mass 2 conditions, we observed short-circuit current densities of 14–15 mA cm^{-2}, open-circuit voltages (V_{oc}) of 0.59–0.61 V and photoelectrode efficiencies of 6.5–7.0%, which are quite excellent properties for an n-InP surface-barrier device. The modest fill factors (rectangularity of the I–V curves[10]) arise from residual concentration overpotentials and uncompensated series-resistance losses in the test-cell design[11], both of which can be minimized by optimal electrode-electrolyte configurations[12]. The I–V properties at lower light levels (<10mW cm^{-2}) show (Fig. 2*b*) that improved fill factors are obtained when

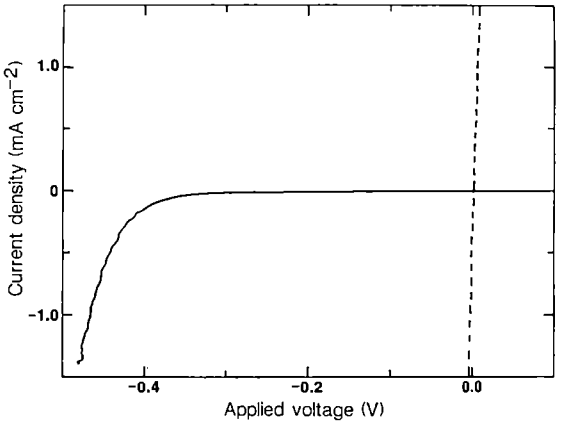

FIG. 1 Dark I–V properties of the n-InP/CH_3OH–0.20M Me_2Fc–0.10M Me_2Fc^+ semiconductor/liquid junction (solid line) and of the n-InP/Au Schottky barrier (dashed line). Negative voltages are forward biases and anodic currents are positive. n-InP Schottky barriers were made by filament evaporation of the metal in a vacuum of base pressure $<5 \times 10^{-6}$ torr.

these losses are minimized. Photoelectrode stability was confirmed for charge passed $>10^2$ C cm^{-2}, which is in agreement with previous rotating ring-disk studies on a related n-InP/CH_3OH interface[13] and indicates that the observed photovoltages are not an artefact of a corrosion potential acting in series with the regenerative cell photovoltage.

The I–V properties of the n-InP/CH_3OH junction were also found to be sensitive to the redox potential of the electrolyte. Figure 3 depicts the I–V behaviour of n-InP/$Me_{10}Fc^{+/0}$–$LiClO_4$–CH_3OH–tetrahydrofuran and n-InP/$CoCp_2^{+/0}$–$LiClO_4$–CH_3OH junctions. The $CoCp_2^{+/0}$ system has the most negative redox potential (that is, the Fermi level that is closest to the vacuum level), and this solution produced poor rectification properties with n-InP samples. The n-InP/$Me_{10}Fc^{+/0}$ system had higher V_{oc} values and better dark-rectification properties, in accord with expectations for an unpinned semiconductor surface Fermi level over this range of solution redox potentials. This behaviour is consistent with dark-current cyclic-voltammetry studies of related n-InP/CH_3CN interfaces[14] but contrasts with the low barrier heights found for various n-InP/metal contacts[5–9].

The three metallocenes used in this study are sufficiently similar in their charge-carrier capture properties (that is, their solvent reorganization energies and heterogeneous electron-capture rates)[15–18] that the most reasonable explanation for the differences between Figs 2 and 3 is a large change in the barrier height of the n-InP/liquid contacts with changes in the value of the solution redox potential. The changes in the reverse saturation current are in good agreement with expectations based on increasing barrier heights with more positive solution redox potentials. Furthermore, the data for the lowest J_0 system, n-InP/CH_3OH–$Me_2Fc^{+/0}$, cannot readily be rationalized by assuming that the barrier height of the semiconductor/liquid contact is pinned at the 0.3–0.5 V value of n-InP semiconductor/metal junctions. Using a 0.4 V barrier height in the thermionic-emission expression[5–9,19], we calculate that $J_0 = 0.16$ A cm^{-2} for the Schottky contact; thus, J_0 for the liquid contact would have to be decreased by over eight orders of magnitude to yield the observed dark I–V properties of Fig. 1 and to produce the V_{oc} values depicted in Fig. 2.

To explain the very low value of J_0 for the n-InP/$Me_2Fc^{+/0}$

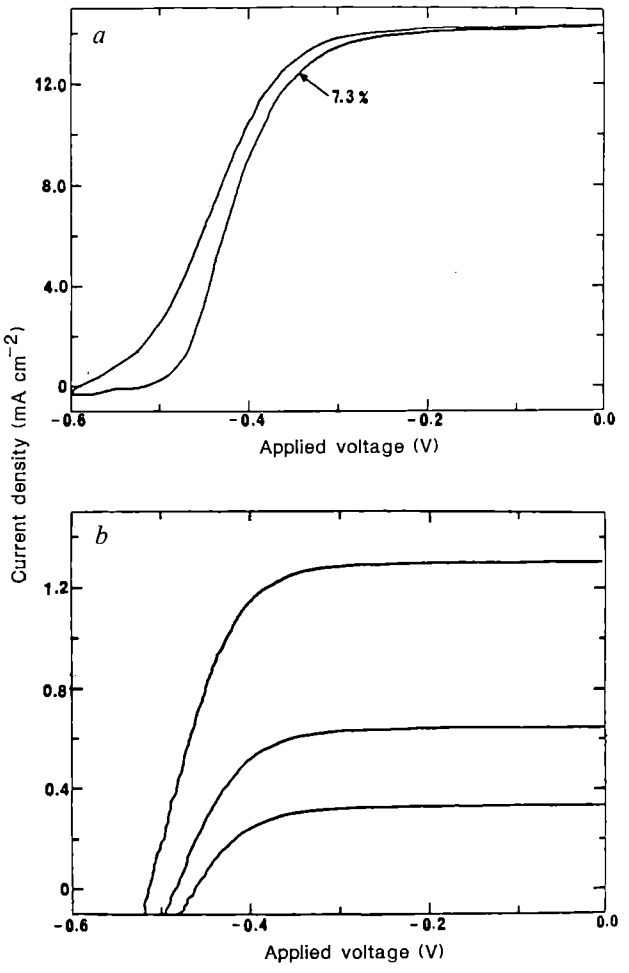

FIG. 2 *a*, I–V properties of the n-InP/CH_3OH–1.0M $LiClO_4$–0.20M Me_2Fc–0.5mM Me_2Fc^+ junction under Air Mass 2 conditions (62 mW cm^{-2}). The electrode was mirror-finished (100)-oriented n-InP and the solution was magnetically stirred. The CH_3OH was distilled from Mg and stored over 3-Å molecular sieves. The $LiClO_4$ was fused *in vacuo* at 280 °C. Illumination was provided by an ELH-type 3,200-K, 100-W tungsten–halogen lamp[3]. The photoelectrode properties were obtained under potentiostatic control relative to a reference electrode at the nernstian potential of the cell ($E = 0.15$ V relative to SCE). The hysteresis between forward and reverse direction scans was largely due to mass-transport limitations of the ions in the solution. The energy conversion efficiency of this particular interface was 7.3%. *b*, I–V properties at lower photocurrent densities showing the improved I–V behaviour at lower concentration overpotentials and series-resistance losses.

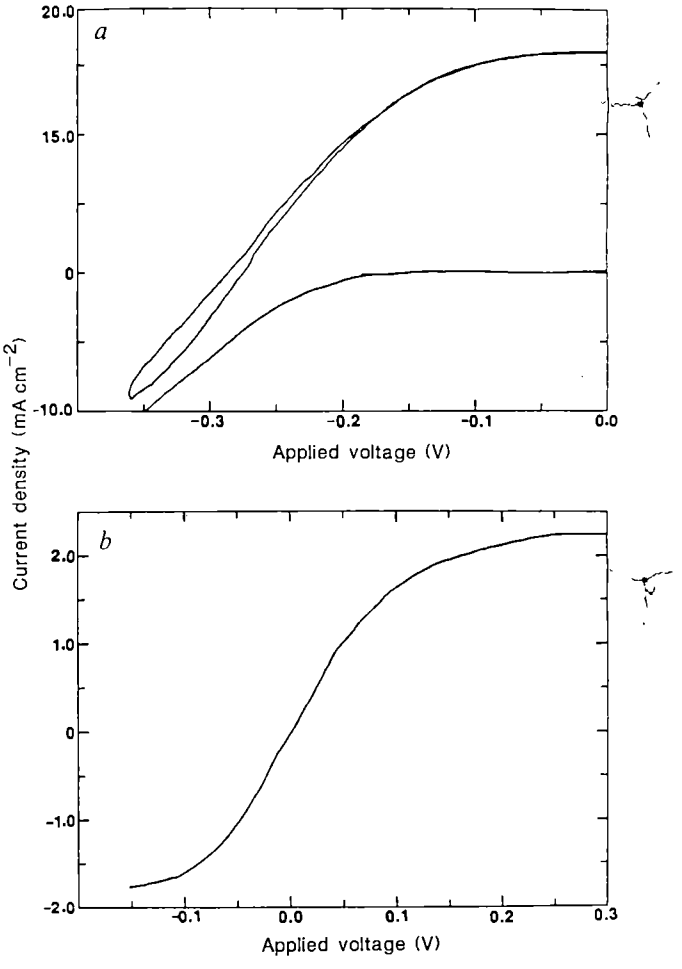

FIG. 3 *a*, I–V properties of the n-InP/CH_3OH (75%)-tetrahydrofuran (25% by volume)–0.50M $LiClO_4$–40.0mM $Me_{10}Fc$–0.5 mM $Me_{10}Fc^+$ system ($E = -0.107$ V relative to SCE) in the dark (lower curve) and under illumination. (Note the scale change on the abcissa as compared to Figs 1 and 2.) *b*, I–V properties of the n-InP/CH_3OH–1.0M $LiClO_4$–20.0mM $CoCp_2$–1mM $CoCp_2^+$ ($E = -1.10$ relative to SCE) interface in the dark. Compared to the I–V data in *a* and Fig. 1, this system shows much higher dark currents and poorer rectification. I–V curves under Air Mass 2 illumination exhibited very low (<10 mV) V_{oc} values.

system, it is necessary to postulate either a large tunnelling barrier at the n-InP/CH_3OH interface, an anomolously low cross section of electron capture for the Me_2Fc^+ ion or a substantially increased barrier height for the liquid junction. The presence of a thick, insulating tunnelling barrier is ruled out by the low series resistance for charge transfer evident in the $I-V$ data of Figs 1 and 2. Also, X-ray photoelectron spectra indicate that n-InP photoelectrode surfaces that have been operated in the CH_3OH-$Me_2Fc^{+/0}$ electrolyte possess $\leq$1–2 Å of oxide overlayers, which is consistent with the low levels of oxide observed by other workers after emersion of InP from other liquids[20]. Postulating a low electronic cross section of carrier capture for the Me_2Fc^+ ion would be in direct conflict with the excellent dark $I-V$ behaviour in Fig. 1 and is also in contradiction to the rapid, reversible electron transfer rates exhibited by ferricenium ions at numerous electrode surfaces[15–17]. For liquids, some suppression of electron injection rates might be expected because of the necessity for solvent reorganization around the acceptor ion and the relatively low concentration of acceptor states[21]. A discrepancy of 10^8 in exchange current is, however, far too large to be explained in this fashion[21]. We thus conclude that the effective barrier height for majority-carrier transport in the n-InP/$Me_2Fc^{+/0}$-CH_3OH semiconductor/liquid system is much larger than that obtainable for n-InP semiconductor/metal Schottky barriers.

Additional information regarding the recombination processes at the n-InP/CH_3OH interface can be obtained[2] from plots of V_{oc} against temperature T. Such plots for the n-InP/CH_3OH-$Me_2Fc^{+/0}$ system (220–300 K) were extremely linear (correlation coefficient >0.995) and yielded an extrapolated intercept ($T=0$ K) of 1.2 V. This value is much higher than the 0.4-V intercept expected for electron exchange processes over a 0.4-V barrier height[10,19]. Also, at low temperature (234 K) and higher light intensity (~300 mW cm^{-2} of ELH-type W-halogen illumination), we observed V_{oc} values >0.81 V. These data, combined with the room temperature $I-V$ data of Fig. 1, clearly indicate that restrictions on photovoltaic performance and on junction leakage predicted by Fermi-level pinning at Schottky barriers do not apply to n-InP/CH_3OH liquid interfaces. It is this difference between the interfacial properties of liquid/semiconductor and metal/semiconductor junctions that enables the construction of efficient photoelectrochemical cells from semiconductors such as n-GaAs and n-InP, even though these materials have been shown to yield inefficient Schottky-barrier photovoltaic devices.

The n-InP system provides a key comparison in the III-V family of semiconductors because it is well documented that the surface Fermi level is pinned very near (0.3–0.5 V) the conduction band for a variety of metal contacts[5–9]. This implies that extremely poor InP junction behaviour should be obtained for all such systems. Previous reports have observed inferior $I-V$ properties of n-InP/liquid contacts[22–24] and differential capacitance measurements have been interpreted as indicating a high density of surface states pinning the Fermi level of n-InP/liquid junctions[25]. By contrast, our data on n-InP/liquid systems clearly show that excellent photoelectrochemical-cell systems can be obtained despite the limitations of the solid-state devices, and that the $I-V$ behaviour of the semiconductor/liquid devices can be extremely responsive to the electrochemical potential of the contacting phase. In fact, the $I-V$ properties of the mirror-finished, (100) n-InP/CH_3OH-$Me_2Fc^{+/0}$ semiconductor/liquid junctions in Fig. 2 are comparable to those obtained from mirror-finished, (100) n-GaAs interfaces[26], which have yielded photoelectrochemical-cell efficiencies of >10% after optimization of surface etching and electrochemical-cell configurations[2,26]. In conjunction with the recent studies of passivation of III-V surfaces at interfaces with non-conductors[27–29], the observation that efficient energy conversion systems can be obtained with n-InP/liquid contacts demonstrates that semiconductor/metal barrier-height measurements are not a reliable guide to the $I-V$ behaviour of III-V semiconductor/liquid interfaces and that new options are available for the utilization of semiconductor/liquid systems in energy conversion applications. □

Received 21 February; accepted 13 July 1989.

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ACKNOWLEDGEMENTS. We thank Dr N. Newman, Prof. W. Spicer and Dr S. C. Pyke for supplying some InP samples and for discussions. We also thank B. J. Tufts for performing photoemission experiments. This work was supported in part by the Office of Naval Research. N.S.L. also acknowledges support as a Dreyfus Teacher-Scholar and as an A. P. Sloan Foundation Fellow. This is contribution number 7882 from the Division of Chemistry and Chemical Engineering.

Direct measurement of the diffusive sublayer at the deep sea floor using oxygen microelectrodes

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THE diffusive sublayer is the region of fluid next to a solid surface, where turbulence is suppressed and molecular diffusion dominates transport of solutes. Diffusive impedance of solute exchange across the benthic sublayer in the deep sea can limit the rates of some diagenetic reactions in the sediment. We present the first direct *in situ* measurements of the thickness of the diffusive sublayer for dissolved oxygen in the deep sea. The positions of 17 oxygen microelectrode profiles relative to the visible sediment/water interface reveal that the sublayer is 0.5–1.5 mm thick, with measurements ranging to 3.5 mm. The sublayer reduces the diffusive flux of oxygen into the sediments by ~10% in these environments. Also, the diffusive flux of isotopically light carbon through the sublayer should cause the ^{13}C content at the interface of typical deep-sea sediments to be ~0.1‰ lighter than the bottom-water value, setting a limit on the precision of the record of past bottom-water given by the carbon isotope composition of the shells of benthic foraminifera.

Sedimentary diagenetic reactions that may be influenced by the diffusive sublayer include the dissolution of $CaCO_3$ (refs 1-3), the growth of manganese nodules[4] and oxic respiration in sediments underlying regions of high productivity[5]. Also, it is important to know the thickness of the diffusive sublayer for interpretation of benthic fluxes measured directly using the flux-chamber method[6,7].

Measurements of the sublayer thickness for O_2 in a laboratory flume ranged from 1.1 mm for a bulk flow velocity of 1-2 cm s^{-1}, to 0.5 mm for a velocity of 10-20 cm s^{-1} (ref. 5). The thickness of the diffusive sublayer above flat plates of dissolving alabaster placed on the ocean floor has been estimated to be ~1.8 mm (ref. 8, see ref. 4 for reinterpretation). These measurements are in accord with hydrodynamic estimates[9] ranging from 0.5 to 2.0 mm, for a friction velocity of the bottom water, U_*, in the range 0.05-0.3 cm s^{-1}, which is typical of the ocean bottom[10]. The plate dissolution experiments and the hydrodynamic estimates both assume unstratified fluid flowing above a smooth flat wall, but the diffusive sublayer over deep-sea sediments could be affected by gradients in viscosity or density caused by flocculent sediment above the interface (for example, see ref. 11) or by surface topography[5,12].

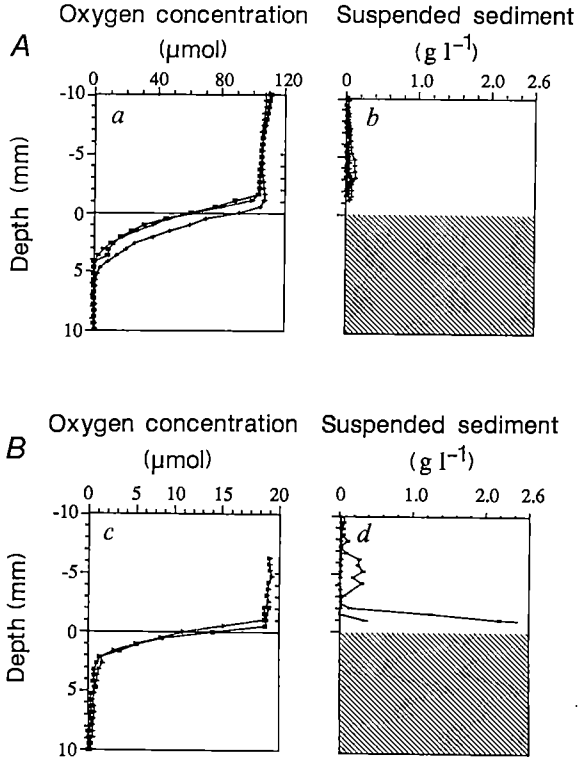

FIG. 1 Sediment/water interface oxygen concentrations and bottom-water transmissivity profiles measured *in situ*, *A* on the shelf and slope of Washington State and *B* in the Santa Catalina Basin. *a* (top and bottom), Representative oxygen profiles from one deployment in each region. The oxygen microelectrodes were similar to those reported in ref. 22. Their sensitive tips are 0.05 mm in diameter, and their response times are ~1 min. The electrodes were linear at oxygen tensions of 2-150 μmol l^{-1} and the output decreased <2-5% in response to the transition from vigorous stirring to stagnant conditions (see ref. 3 for further discussion), a much smaller change than was observed across the diffusive sublayer. *b* (top and bottom), All the near-bed turbidity data from multiple deployments in each region. The transmissivity sensor consisted of two fibre-optic cables, 1 mm in diameter, facing each other, 8 mm apart. The extinction coefficient for suspended surface sediment was measured in the laboratory. The sensor has a detection limit of 0.1 g l^{-1} or better of suspended sediment *in situ*. No increase in near-bed turbidity was detected over the sandier sediments of the Washington Shelf (*b*, top). Turbidity values found above sediments in the Santa Catalina Basin (*b*, bottom) are three orders of magnitude greater than reported values in the nepheloid layer hundreds of metres away from the bed in the California Borderlands[23].

In October 1987 and November 1988 we measured the thickness of the sublayer for O_2 *in situ* by positioning a microelectrode profiling system[3] at the sediment surface in the Santa Catalina Basin[13,14] using the submersible research vessel *Alvin*. The profiler was also deployed outside a remote benthic tripod on the shelf and slope of Washington State[15] in June 1988. In all locations the concentration of dissolved oxygen decreased from bottom-water values to unmeasurable levels within 4-8 mm (Fig. 1). We estimate the depth at which the electrode crossed the sediment/water interface by mounting 2-mm diameter dowels, painted with high-contrast white and black bands, next to each electrode and monitoring, by video recording, the penetration of the dowels into the sediment (Fig. 2). The thickness of the sublayer can be estimated with a precision of the ~0.5-mm vertical spacing of the oxygen measurements. A fibre-optic transmissivity sensor was installed for some of the deployments (June and November 1988), which enabled us to measure bottom-water turbidity as close as 1 mm above the bed.

We determined the decimetre-scale topography at each deployment site by using the relative depths at which each of the four electrode tips penetrated the sediment. If the surface slope of the site exceeded a few per cent, and the measuring stick was positioned uphill or downhill from its electrode, the sublayer thickness estimate may have been biased and we excluded it from consideration. In each of the rejected profiles, the sticks happened to be placed uphill of the electrodes, potentially underestimating the sublayer thickness. The remaining estimates are summarized in Fig. 3. The thickness of the sublayer is operationally defined as the vertical distance between the lowest depth at which bottom-water O_2 was detected and the location of the visible sediment/water interface.

The distribution of sublayer thickness measurements from each region varied by ~±0.5 mm. Much of this variation occurs in profiles ~10 cm apart, from the same deployment. The reproducibility of the measurement at each location to within the uncertainty of the technique implies that sediment topography on the scale of the distance from the stick to the electrode tip (~1 cm) was not large enough to affect our results significantly. An attempt to interpret the ±0.5-mm variation in terms of environmental parameters would, however, probably be misleading.

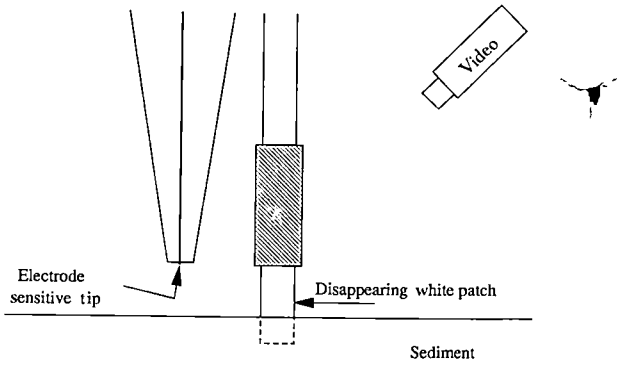

FIG. 2 The method of finding the sediment/water interface for the oxygen profiles. The spatial relationship between the white-dark boundary of each rod and its corresponding O_2 electrode tip was used to locate the visible sediment/water interface, independently for each electrode, using video recording. The camera was placed at an angle of 50° from the vertical. The light was focused by a lens of focal length 8.5 mm onto a charged-coupled sensor that was 8.8×6.6 mm. The vertical resolution of the recorded was ~0.25 mm per pixel with the camera placement used. Laboratory tests confirmed that the video camera was able to resolve the disappearance of the tip to <0.5 mm. In sediments with a flat interface, positioning the stick at a horizontal distance of 1 cm from the electrode gave, on average, the same results as using a distance of 0.3 cm, suggesting that disruption of the bottom current by the stick did not significantly bias the results.

There does seem to be a systematic difference between the results from the single deployment at Santa Catalina Basin in October 1987 and the other observations, including data from the same location one year later (Fig. 3). We have detected no experimental flaws in this deployment. If the thicker sublayer were caused by lower bottom-water turbulence, the flow velocity would have to be roughly five times lower than in the other observations[9], which was not supported by the drift rates of suspended particulates in the video records or by visual observations from *Alvin*. The observations could also be explained by the presence of a 1- to 2-mm-thick layer of flocculent material at the interface. No such layer was visible from *Alvin*, however, and the turbidity sensor was not deployed at this time, so this hypothesis cannot be verified.

We observed a near-bed increase in turbidity in the Santa Catalina Basin one year later, in contrast to results from the sandier sediments on the Washington shelf (Fig. 1). In spite of the difference in suspended particulate loads, the diffusive-sublayer thickness data from the two locations are probably not significantly different. If the solid material in the turbidity layer was not attached to the bed in any way, it should have increased the suspension viscosity by ~2% g l^{-1} of suspended sediment[16]. The diffusive-sublayer thickness is expected to increase proportionally (1–5% total change for the turbidity observed)[9].

Except for the first Santa Catalina Basin cruise results, the diffusive sublayer is ~1 mm thick, in accord with hydrodynamic prediction. Although the precision of these measurements is not as great as obtained by the plate-dissolution method[8], this study shows that the assumption of unstratified fluid flowing over a smooth, flat wall is not fundamentally in error for the sublayer over fine-grained deep-sea sediments. This assumption is integral to sublayer thickness estimates based on the dissolution experiments and on hydrodynamic theory[9].

Diffusive-sublayer limitation of solute-controlled sediment reaction rates is most important if the characteristic reaction time of the solute in the pore water is shorter than the solute transport time across the sublayer. Thus, the sublayer exerts greater control of the sedimentary oxygen flux as the penetration depth of oxygen into the sediment decreases, namely, in areas of high carbon rain or low bottom-water oxygen concentration. Our calculations indicate that, for O_2-penetration depths typical of this data set, a diffusive sublayer 1 mm thick decreases the oxygen flux by ~10% relative to the case of no sublayer (see Fig. 4). Limitation of the oxygen flux into the sediments would act to enhance the preservation of organic carbon in these environments, if oxic respiration is more effective than anaerobic metabolism[17].

The sublayer gradient in the carbon-isotope ratio of dissolved inorganic carbon (DIC) due to the flux of isotopically light metabolic CO_2 may pose limits on the precision of benthic-foraminifera tests as recorders of past bottom-water ^{13}C content (Fig. 4). For organic carbon rain rates typical of the pelagic sediments where palaeoceanographic foraminifera samples are usually taken (10–50 μmol C cm^{-2} yr^{-1} (ref. 3)), there should be a 0.1–0.3‰ transition in the sublayer between the δ^{13} DIC value in bottom water and at the interface. Because benthic forminifera are small enough to live within the sublayer[18], they could record the δ^{13} DIC of this region. Some foraminifera species have been observed to position themselves outside the sublayer on stalks[19]; unless the entire population does this

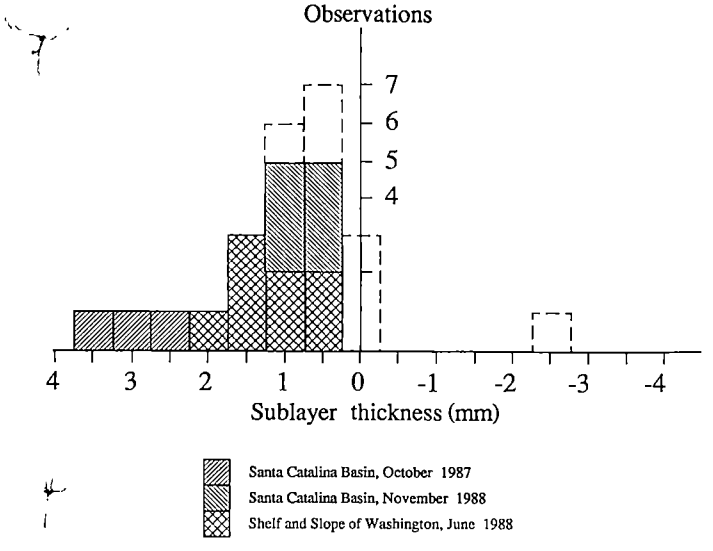

FIG. 3 Summary of 17 *in situ* determinations of the thickness of the diffusive sublayer. The observations indicated by dashed lines are probably an artefact of surface topography (see text). The study confirms that the thickness of the diffusive sublayer is usually 0.5–1.5 mm.

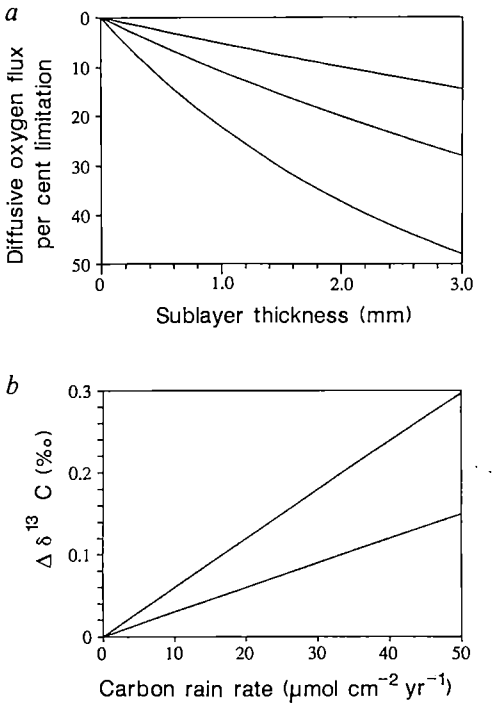

FIG. 4 (*a*), The response of the diffusive oxygen flux into the sediments to thickness of the diffusive sublayer for O_2 penetration depths (with no sublayer) of 2.5, 5.0 and 10.0 mm (lower, middle and upper curves, respectively). As the sublayer thickness increases oxygen penetrates less deeply into the sediments and there is less organic carbon within the oxic zone available for reaction, reducing the oxygen flux into the sediments. The steady-state profile of oxygen is governed by the processes of diffusion and reaction[3,24]. In the sediment, the reaction rate is modelled as first-order with respect to carbon, and zero-order with O_2, until O_2 is depleted. The reaction rate within the sublayer is zero. The organic carbon concentration with depth is assumed to be proportional to the volume fraction of solids in the sediment (the complement of the porosity profile, derived from measured resistivity data and fit to an exponential[3,25]). Assuming no sublayer, and specifying the penetration depth of O_2 into the sediment, the kinetic rate constant for organic degradation is determined by equating the diffusive flux of oxygen into the sediment with the integrated degradation rate of the organic material within the oxic zone. The rate constant is held fixed as the sublayer thickness is increased to predict the effect of the sublayer on the benthic O_2 consumption rate. The results presented do not include the effect of the porosity on the diffusion coefficient, the effect of a potentially varying solid fraction of labile organic carbon with depth, or the possibility of O_2 reacting with dissolved species. *b*, The difference between the $\delta^{13}C$ of DIC at the sediment/water interface and in the bottom water, $\Delta\delta^{-3}C$, plotted as a function of the particulate-organic-carbon rain rate for diffusive-sublayer thicknesses of 0.5 and 1.0 mm (lower and upper curves, respectively). Gradients in $\Delta\delta^{13}C$ expected within the sublayer were calculated by extrapolating pore-water $\delta^{13}C$ data[26,27] to the sediment/water interface.

uniformly, however, the gradient of δ^{13} DIC within the diffusive sublayer could easily result in the 0.1‰ variation in the carbon isotope ratio observed[20,21]. □

Received 11 April; accepted 17 July 1989.

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ACKNOWLEDGEMENTS. We thank Dr A. Devol for assistance with field work, P. Hill, C. Reimers and B. Boudreau for comments, and P. Kauffmann, for the design and construction of the data logger and transmissivity sensor.

Distortions, rotations and crustal thinning at ridge–transform intersections

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WHEN an oceanic ridge intersects a transform fault, the newly formed oceanic crust may be severely distorted, frequently exhibiting major rotations of fault blocks[1–4]. Such effects typically extend over a 10-km-wide strip parallel to the transform. By contrast, studies of contemporary oceanic transforms suggest that deformation is restricted to a single fault trace within the transform domain[5–9]. This apparent inconsistency can be reconciled if the rotations occurred at the ridge–transform intersection during accretion of the crust. Here I develop a kinematic model that considers the effect of an ephemeral period of stretching on a region that broadens as the transform is approached along the ridge. The strains calculated from this model indicate extension at the ridge, simple shear at the transform and composite strains in the region of distributed deformation. The model predicts very rapid rotation rates (100° in 10^4–10^5 yr). The implications for the topography and structure of ridge–transform intersections are considered, and the alternating periods of extension by stretching and by intrusion are illustrated by reference to contemporary transforms on the Mid-Atlantic Ridge. The model may also explain the common occurrence of cross-cutting dykes near transform faults in ophiolites.

Large clockwise rotations of a dextral dyke swarm have been observed to the south of the dextral-slipping Tjournes fracture zone in Iceland[1]. Heterogeneous simple shear has affected a 10-km wide strip, with rotations locally apparently >90° (Fig. 1*a*). A similar effect is noted on the Troodos ophiolite of Cyprus to the north of the dextral-slip Arakapas transform fault, where clockwise fault-block rotations have been confirmed by palaeomagnetic studies[2,3] (Fig. 1*b*). Again, the region affected is about 10 km wide. In the eastern part of the complex a ridge jump has preserved crust formed at the non-transform corner of the ridge–transform intersection[3] that does not exhibit significant rotations about vertical axes. Large rotations and stretching lineations have been recorded from deeper structural levels at the Newfoundland coastal complex ophiolite[4] (Fig. 1*b*), up to 5 km from a fossilized oceanic transform fault. In these three examples where transform faults are exposed in ophiolites, the crustal sequence adjacent to the fault has been rotated, locally >90°. In the examples from both Iceland and Cyprus, the rotated material is deformed by brittle faulting outside the transform domain, the domain being defined by distinctive escarpments in each case.

In contemporary oceanic transform faults, strike-slip deformation seems to be confined largely to the transform zone, and active tectonism is restricted to a very narrow belt less than 1 km wide[5–9]. Although the width of the plate-bounding shear zone may increase with depth, it is not clear how this could develop the rotations in the oceanic crust of the transform walls.

An appealing alternative is that the rotations occurred very close to the plate boundary, at the ridge–transform intersection, when it was still hot and weak enough for brittle failure to occur. Recent studies of the structure of intermediate- to slow-spreading oceanic ridges suggest that during periods of reduced magma supply, in the absence of a magma chamber[10–13], extension is accommodated by normal faulting. Seismic reflection profiles from the fast-spreading East Pacific Rise[14] show that a magma chamber is not in evidence next to the Clipperton transform. Magma chambers thus may frequently be absent at ridge–transform intersections and stretching may be a common phenomenon in this environment. This is supported by the microseismicity at ridge–transform intersections[15,16]. Earthquakes are frequently scattered in the transform corner, rather than delineating a narrow plate boundary, which suggests that brittle deformation may be distributed over a broad region at the ridge–transform intersection.

My model explores some of the kinematic implications of a period of extension by faulting at a ridge–transform intersection, in terms of the induced strains and rotation rates. Two rigid plates at a ridge–transform intersection are defined and deformation is assumed to be restricted to the area between them. A simple two-dimensional velocity structure for points in the deforming region is assumed, such that

$$u = kxy - u_1/2 \quad (1)$$

$$v = 0 \quad (2)$$

(see Figs 2 and 3). Plate A moves at $u = -u_1/2$ and plate B, with a boundary defined by $u_1 = kxy$, moves at $u_1/2$. This velocity structure was chosen as the simplest that gave $u = -u_1/2$ on both $x = 0$ and $y = 0$ and that gave an area of distributed deformation that broadened as the transform was approached.

This simple model illustrates the effects of a short-lived stretching event. At the ridge away from the transform (at the top of Fig. 2*c*) deformation is essentially pure shear producing vertical plane strain. Along the transform, simple shear develops horizontal plane strain. The strains are transitional between these end conditions. Strains with axial ratios of 1:2:10, similar to those described at the Newfoundland coastal complex, were generated after only 100,000 years of stretching across a 2-km zone[4]. Styles of brittle deformation on the Troodos ophiolite

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are also consistent with the strains developed here, with subsidiary strike-slip faults largely confined to the transform corner, and normal faults dominating in the non-transform corner[3].

The rotations of strain ellipsoids are equivalent to those of passive markers. Using the velocity gradients, the rotation rate can be deduced for rigid particles within the flow. For many continental situations this rate is also appropriate for fault-blocks at the surface[17].

The velocity vector $\mathbf{v}$ can be defined such that

$$\mathbf{v} = (u, v, w) = (kxy - u_1/2, 0, w) \quad (3)$$

so that the rate of rotation, or vorticity, ω, is given by

$$2\omega = \nabla \mathbf{x} v = \left(-\frac{\partial w}{\partial y}, \frac{\partial w}{\partial x}, kx\right) \quad (4)$$

Thus $kx/2$ represents the instantaneous rotation rate about a vertical axis (ω_z). This implies that ω_z is proportional to the width of the deforming zone parallel to the transform, and is constant at any point along the transform. The modification of ω_z with time depends on how the boundaries of the deforming zone are considered to evolve. Using realistic dimensions (a ridge 1–2 km wide and 10–20 km from the transform) and spreading rates (5×10^{-2} m yr^{-1}–10^{-1} m yr^{-1}), rotation rates of 10^{-4}–10^{-5} rad yr^{-1} can be generated, which would produce the ~2-rad rotation observed at the Troodos ophiolite in 10^4–10^5 yr. Large rotations can occur within the dyke-intrusion zone, so that cross-cutting dykes should be common. These are observed close to fracture zones in ophiolites[1,3,4].

A period of amagmatic stretching requires lithospheric thinning, which will generate the deep nodal basin frequently associated with ridge-transform intersections. The rate of thinning is inversely proportional to the width of the deforming zone and so will increase with distance from the transform. This thinning will be balanced by material being injected laterally from a magma chamber some distance from the ridge. The deepest part of the nodal basin will thus be expected to lie between the

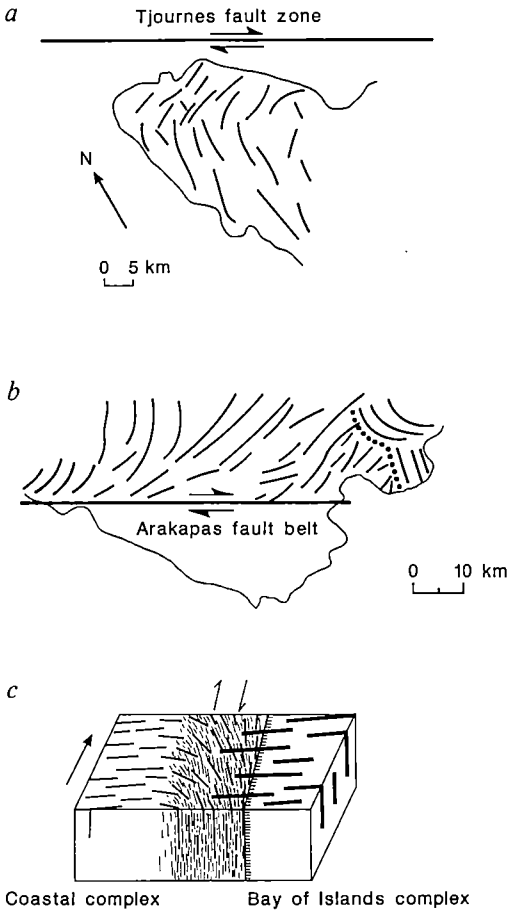

FIG. 1 Dyke trends at exposed fossil fracture zones. *a*, Tjournes fracture zone, north central Iceland[1]. *b*, Arakapas fossil transform fault, Troodos ophiolite, Cyprus (east–west trend)[3,22]. Note the major change in dyke strike to the east of the ridge-jump boundary (dotted line). Palaeomagnetic results[2,3] indicate that dykes to the west of this boundary have been rotated by ~100° clockwise about steeply inclined axes, whereas those to the east have not. The area to the west is believed to have formed at the transform corner of a ridge-transform intersection, and those to the east, at the non-transform corner. *c*, Schematic diagram illustrating dyke trends on the Bay of Islands and coastal complex ophiolites in Newfoundland, in their preobduction setting[4]. Deformation of the coastal complex is by ductile shear. The coastal complex is believed to have formed at a transform corner, and the Bay of Islands at a non-transform corner, of a ridge-transform intersection.

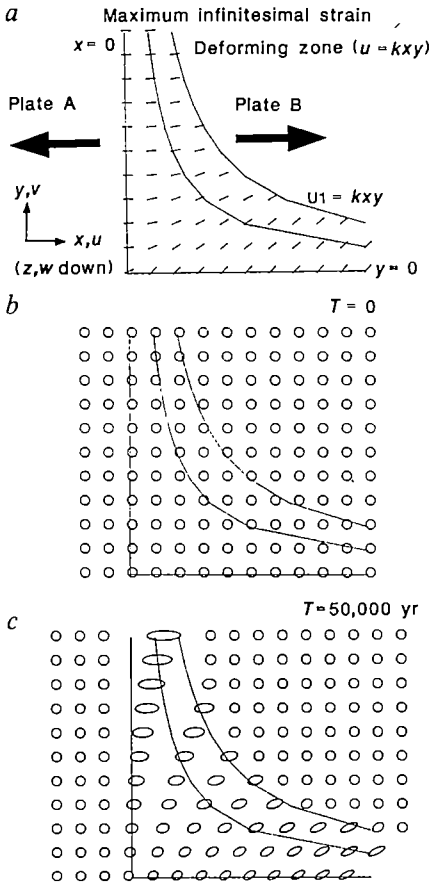

FIG. 2 Model for effect of stretching on the ridge-transform intersection. Displacement vector $x\mathbf{i}$ is defined as parallel to the transform, $y\mathbf{j}$ perpendicular to the transform and $z\mathbf{k}$ vertical down. The corresponding velocity components are u, v, w. Plate A moves at $u = -u_1/2$, $v = 0$ and is defined by $y = 0$ and $x = 0$. Plate B moves at $u = u_1/2$ relative to plate A, and is defined by the boundary $u_1 = kxy$. The central line $u_1/2 = kxy$ remains fixed. The plates are considered rigid; deformation is restricted to the region between them and is described by the velocities $u = kxy - u_1/2$ and $v = 0$. *a*, Orientation of maximum infinitesimal strain axes. Dykes will be intruded perpendicular to these axes and thus will make a smaller acute angle to the transform at the transform corner than at the non-transform corner. This relationship is observed on the Troodos ophiolite[3]. *b*, Initial horizontal unit circles (radius = 1 m) and *c*, orientation after 50,000 years of stretching. Pure shear (extension perpendicular to the ridge) develops vertical plane strain at the ridge (top left), and simple shear (strike-slip) develops horizontal plane strain at the transform (bottom right). The strains are transitional between these end conditions. This is consistent with the orientation of brittle fractures on the Troodos ophiolite.

magma chamber and the transform fault. The cumulative thinning, however, is inversely proportional to the amount of new material injected, and will be greatest close to the transform, as is commonly observed on seismic refraction profiles[16,18]. Such a thinning process would also explain the occurrence of lower crustal rocks frequently observed at the transform corner[19]. The anomalous uplift of the transform corner which has previously been attributed to serpentinized ultramafics would be enhanced by the increased faulting in the region predicted by this model.

Extension by stretching, illustrated in Fig. 3*a*, is exemplified by the eastern ridge–transform intersection of the Oceanographer fracture and the Mid-Atlantic Ridge[20], which is characterized by a deep nodal basin, largely devoid of sediment fill. The axis of accretion and the principal transform displacement zone are not clearly defined, and the neovolcanic zone is increasingly disrupted by fracturing as the transform is approached. The faults in the non-transform corner are ridge-parallel, whereas those at the transform corner have very variable orientations. Serpentinized ultramafics are exposed on the flanks of the basin.

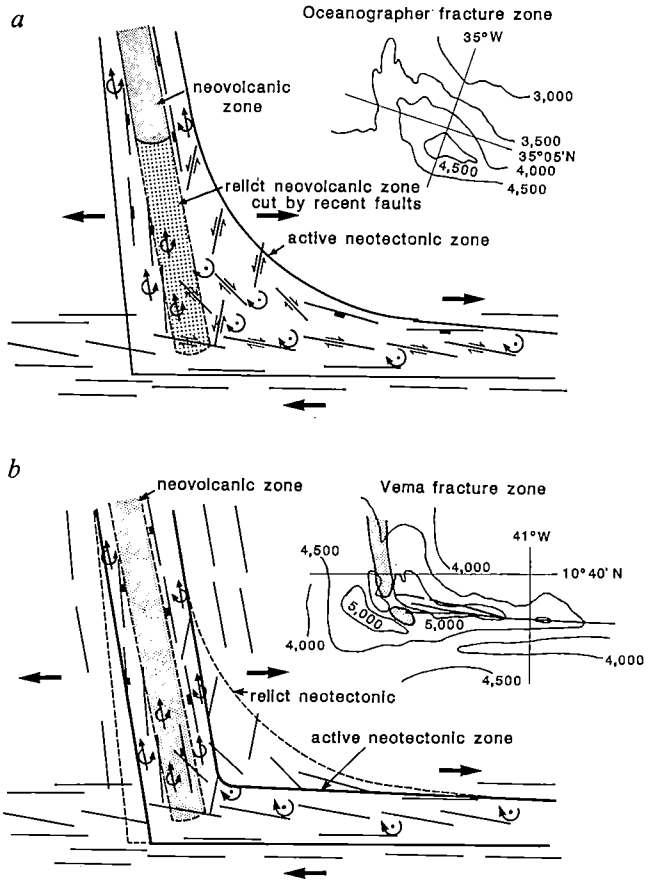

FIG. 3 *a*, Principal features of a ridge–transform intersection undergoing stretching, exemplified by the eastern intersection of the Oceanographer transform and the Mid-Atlantic Ridge (reversed for comparison)[20]. A relict neovolcanic zone is truncated by active tectonism. The principal transform displacement zone is poorly defined within the well developed nodal basin. The model predicts the occurrence of normal faulting at the non-transform corner, and oblique-normal/strike activity at the transform corner. *b*, Extension by intrusion, exemplified by the eastern intersection of the Vema fracture zone with the Mid-Atlantic Ridge (rotated for comparison)[21]. Here, an active neovolcanic zone bisects a relict nodal basin, reflecting rejuvenated magmatic activity and uplift. The principal transform displacement zone is confined to a narrow, well defined trace. Tectonism is limited to a narrow region of normal faulting at the ridge, and of strike-slip faulting in the transform valley. (Depths in m.)

The following phase of rejuvenated neovolcanic activity, and a narrow deforming zone, can be identified in the eastern intersection of the Mid-Atlantic Ridge with the Vema fracture zone[21] (Fig. 3*b*). Here, a volcanically but not tectonically active zone joins a well developed principal transform zone that is tectonically active, the various splays of which occupy a 2-km wide belt. The neovolcanic zone is elevated relative to the deep nodal basin that surrounds it.

The faults observed at ridge–transform intersections are dominantly dip-slip. This may reflect the dominance of vertical tectonism within this region, but does not preclude a small, but significant component of strike-slip movement, sufficient to produce the required rotation. This is the case for the eastern Greece continental graben system, where a rotation of up to 45° has been accommodated by a small component of strike-slip on otherwise normal faults[17].

Ephemeral periods of stretching at ridge–transform intersections would generate the rotations observed in ophiolites. These rotations are probably accommodated by composite normal-strike slip faults in the brittle crust. Rotation rates may be high, and short periods of extreme tectonic activity may be expected. Cross-cutting dykes are a probable consequence of rotation close to the spreading axis. Between stretching events the plate boundaries will be narrower and more clearly defined by ridge and transform segments. Plate movement will be accommodated by dyke intrusion. □

Received 5 June; accepted 20 July 1989.

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ACKNOWLEDGEMENTS. I thank the staff and students of the School of Environmental Sciences at the University of East Anglia, where this work was completed, and particularly F. J. Vine for his supervision. I also thank A. Mazaud, P. Newton, D. Sutton and P. Tucholka.

Accumulations of melt at the base of young oceanic crust

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As hot, solid rock ascends through the mantle, the pressure around it decreases while its temperature decreases approximately adiabatically. During the ascent, the melting temperature decreases faster than the adiabat temperature, and if the initial temperature of the rock is high enough, the rock will begin to melt at the depth where the adiabat crosses the melting-temperature curve. Models of upwelling beneath mid-ocean ridges predict significant melt production over a zone several tens of kilometres wider than the ridge-axis region suggesting that some of the ascending melt must arrive away from the ridge axis. Here

I report ocean-bottom seismograph observations of an uncommon and previously unreported marine seismic phase, which provide strong evidence that melt accumulates in sills at the base of young oceanic crust. The phase results from the conversion of compressional (P) waves to shear (S) waves on interaction with the crust–mantle boundary (the Moho). This phase is rare and very difficult to model, but when visible, it is strikingly prominent and usually appears at unexpectedly great source-to-receiver ranges. The observations imply the presence of bodies of extremely low rigidity, presumably melt sills, at the Moho close to the receiver. Melt may be fairly mobile at the base of the crust, allowing ridges to accumulate melt through a lateral plumbing system at the locally uplifted boundary between the crust and the underlying thermally expanded mantle. Escape of this melt to the sea floor may be responsible for the formation of off-axis seamounts.

The phase due to a P wave in the crust reflected as an S wave from the Moho is designated[1] PmS, and includes lower-crustal-diffracted P waves that are continuously converted to crustal S waves and which extend true PmS beyond its expected geometric range (~20 km). In addition, P waves refracted in the upper mantle and reflected from the underside of the Moho lose energy to S-wave radiation into the crust and mantle and also contribute to the apparent extension of the phase. I use the designation to denote all of these phases. The point of generation of the observed S waves of PmS is a horizontal distance under 4 km from the receiver in typical oceanic crust, even if the source is very distant. In a model with a simple sharp Moho, PmS is a very inconspicuous phase on the horizontal component of sea-floor displacement between the 15- and 20-km range and at a reduced time of ~3.9 s (Fig. 1). The synthetic seismograms show complete sea-floor motions assuming an overlying water half-space and isotropic elasticity. Phase-slowness integrations were

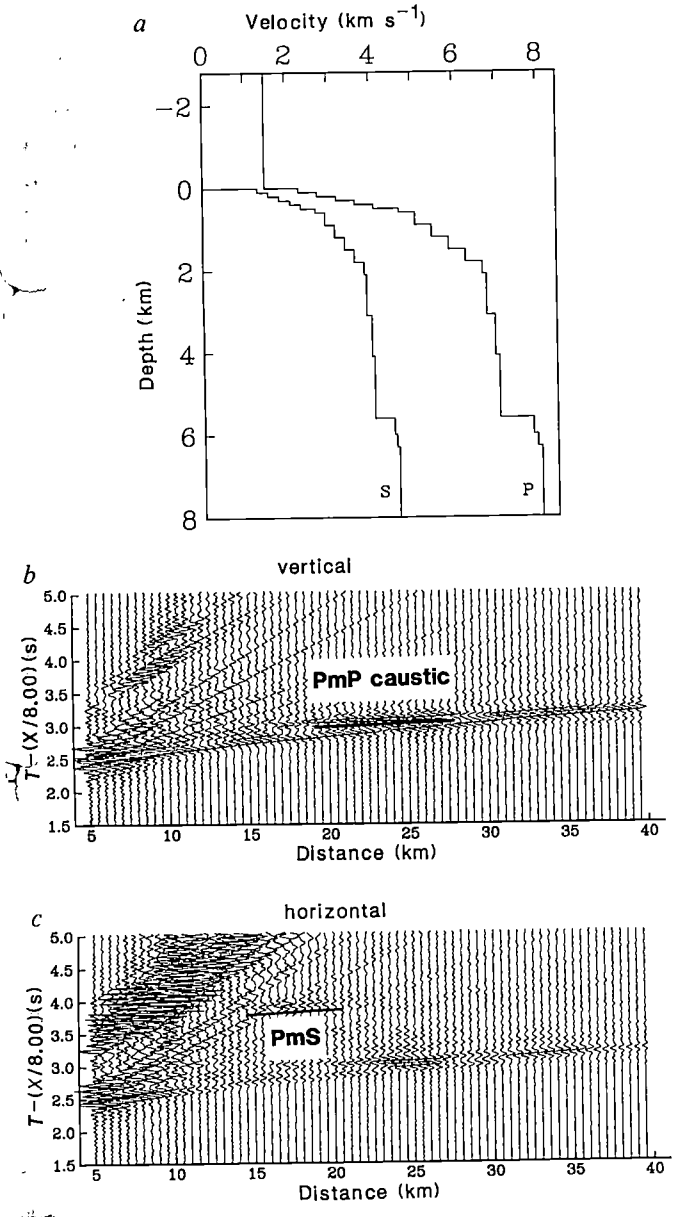

FIG. 1 *a*, Model and *b*, *c*, synthetic seismograms for simple Moho structure. Water depth 2.8 km. P and S velocities shown, densities linearly related to P velocity. PmS phase visible on horizontal (radial) component between 15- and 20-km range at a reduced time of 3.9–4.0 s. The reduced time is $T-(X/8.0)$ where T is the uncorrected time, X is the distance and 8 km s^{-1} is the reducing velocity.

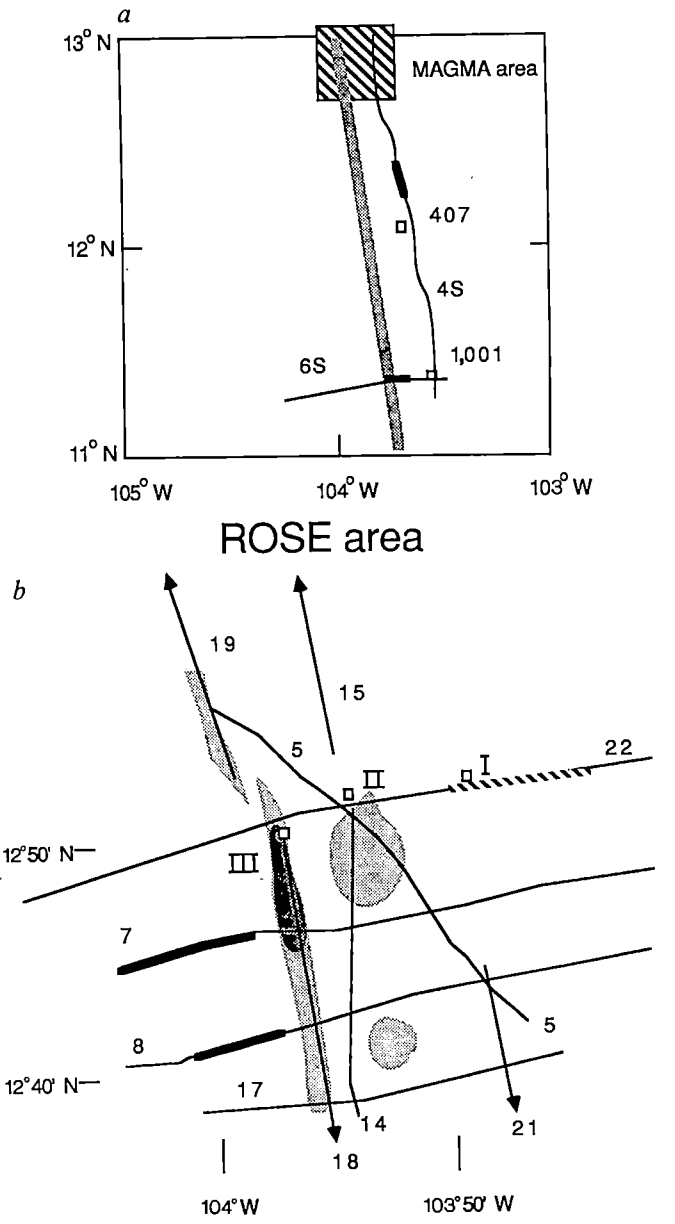

FIG. 2 Simplified location maps. *a*, ROSE area. Grey line is East Pacific Rise. OBS sites are open squares identified by site numbers. Shot lines are numbered, bold segments show the shots that yielded PmS. Hatched square shows approximate coverage of *b*. *b*, MAGMA area. Generally as in *a*. Grey areas are bathymetric highs (East Pacific Rise and off-axis seamounts). Darker grey shows location of known vent activity. Bold lines show shot locations of PmS observations at site II (solid lines) and disturbed propagation to site III (hatched line). Observations of PmS consistent with anomalous Moho between ridge and northern seamount.

calculated from 0.05 to 0.30 s km^{-1} (3.33–20 km s^{-1} phase velocity). Models that have transitional Moho structures that are thicker than a shear-wave half wavelength (~200 m for the middle of the frequency band used here, 5–15 Hz) yield much smaller PmS phases.

A practical difficulty in the observation of PmS is the presence of interfering P-to-S conversions of upgoing P waves just beneath the receiver. These shallow conversions are called Ps (ref. 1), where the s normally signifies that the S part of the path is in the sediments. The Ps waves in the data presented below are from nearly unsedimented regions[2], so the usage is somewhat loose—the conversion actually takes place at the base of the fractured basalt (layer 2a). The Ps phase is seen ~0.2–0.6 s after P; this wide range of delays is largely due to shear-wave anisotropy in the fractured basalt. Although these times are well before the arrival time of PmS (~1.2 s after P), the coda of Ps could make the identification of PmS difficult.

From the simple Moho model, it might be expected that PmS would almost never be observed because it is a weak phase which should be lost in the codas of the P and Ps arrivals. The horizontal-component ocean-bottom seismograph (OBS) data that I have examined largely confirm this conclusion. There are, however, some striking exceptions. A very strong PmS phase is visible on a horizontal (and nearly radial) component of an OBS in the Rivera Ocean Seismic Experiment (ROSE) experiment[3,4] (MIT site 1,001, line 6S). Figure 2*a* shows a simplified location map of the ROSE area. The PmS phase is clearly distinct from the Ps phase and has quite high amplitudes beyond 20 km (Fig. 3*a*). The spatial distribution and sharp onset of the PmS phase rule out the coincidental reinforcement of S wave reverberations to give a false PmS. It is the appearance of PmS well beyond its expected range that argues most strongly that the Moho close to this instrument is unusual. The orthogonal line (4S) to this instrument shows no such phase, indicating that the

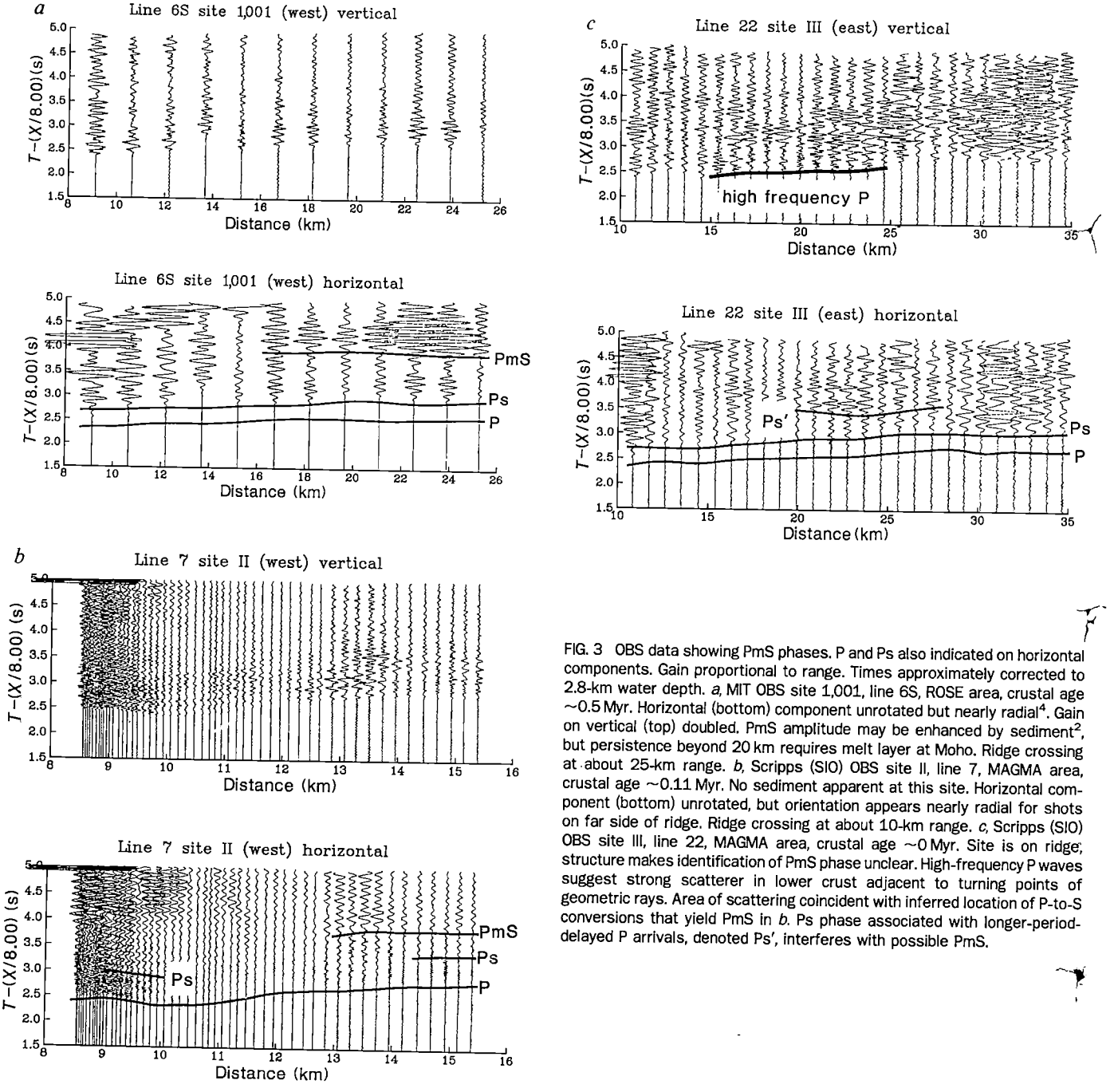

FIG. 3 OBS data showing PmS phases. P and Ps also indicated on horizontal components. Gain proportional to range. Times approximately corrected to 2.8-km water depth. *a*, MIT OBS site 1,001, line 6S, ROSE area, crustal age ~0.5 Myr. Horizontal (bottom) component unrotated but nearly radial[4]. Gain on vertical (top) doubled. PmS amplitude may be enhanced by sediment[2], but persistence beyond 20 km requires melt layer at Moho. Ridge crossing at about 25-km range. *b*, Scripps (SIO) OBS site II, line 7, MAGMA area, crustal age ~0.11 Myr. No sediment apparent at this site. Horizontal component (bottom) unrotated, but orientation appears nearly radial for shots on far side of ridge. Ridge crossing at about 10-km range. *c*, Scripps (SIO) OBS site III, line 22, MAGMA area, crustal age ~0 Myr. Site is on ridge; structure makes identification of PmS phase unclear. High-frequency P waves suggest strong scatterer in lower crust adjacent to turning points of geometric rays. Area of scattering coincident with inferred location of P-to-S conversions that yield PmS in *b*. Ps phase associated with longer-period-delayed P arrivals, denoted Ps', interferes with possible PmS.

conditions at the Moho that make PmS so visible on line 6S are spatially limited. The point of P-to-S conversion is ~22 km from the ridge.

Another instrument in the ROSE area (University of Washington site 407, line 4S north of the instrument) also shows an intense PmS arrival on its horizontal components in the range 20–28 km. The data show no evidence for PmS to the south of the instrument. Here, the point of P-to-S conversion is ~17 km from the ridge.

I have also examined data from the 1982 MAGMA experiment[5,6] for PmS phases. Out of 51 line segments at plausible PmS ranges from the three sites, only two instances of PmS emerged. The two PmS phases appear on unrotated horizontal components in which the raw horizontal data have not been linearly combined to yield motions along an azimuth different from either of the two original orientations of the geophones at site II (Fig. 2*b*) from shots on lines 7 (Fig. 3*b*) and 8. The point of conversion in the MAGMA data lies between an off-axis seamount at 12°50′ N and a segment of ridge that showed active hydrothermal venting one year before the MAGMA experiment[7].

Figure 3*c* shows disturbed propagation to site III from shots on the eastern part of line 22. At ranges between 15 and 25 km, the P arrivals on the vertical component in Fig. 3*c* have a remarkable high-frequency character. Rays at these ranges have turning points at middle-to-lower crustal levels and thus come close to the anomalous Moho, which strongly affects grazing P waves. In general, heterogeneities adjacent to ray paths affect low-frequency waves more than high-frequency waves, and I suggest that this results in the observed high-pass filtering effect. The spectrum of the seismic waves, which is dominated by the fundamental and third harmonic of the bubble pulse, enhances this effect. Therefore, these observations are also consistent with the presence of a strong scatterer at or near the Moho with nearly the same geographical location as the conversion point responsible for the PmS phase at site II.

Of all the instances of PmS presented here, the ROSE observations are the most difficult to explain. Even a very soft serpentine layer with sharp boundaries did not yield PmS at the large ranges seen in the ROSE data. Only the results using a model with a layer of extremely low rigidity at the Moho, interpretable as a sill of partial melt, resembled the data. Figure 4 shows one of these models. The melt layer is 10-m thick and has a P velocity of 4 km s^{-1} and an S velocity of 0.1 km s^{-1}. These values may vary somewhat without much affecting the synthetic seismograms if the rigidity of the melt layer is very low. The PmS phase has the same travel times and distances and is stronger for a 100-m-thick melt layer. The most important result of this modelling is that the PmS phase now extends significantly beyond the 20-km range, in accordance with the ROSE observations. No model without an extremely low rigidity layer produced this feature. Models with smooth, transitional Moho structures, except for interruption by a melt layer, require a thicker (50–100 m) melt layer to yield a strong PmS phase. Velocity gradients below the melt layer can intensify the PmS phase locally.

The amplitudes in the ROSE data are larger than the model predicts. It is most likely that the melt body has only a small lateral extent and that the shear waves are generated on transmission of upgoing mantle P waves. Unfortunately, the propagation effects of this form of heterogeneity are very difficult to model numerically. The absence of melt where the P waves enter the mantle allows stronger P-wave transmission, resulting in stronger long-range PmS than is predicted by the laterally homogeneous model. The Moho structure below the melt layer affects the pattern of amplitudes of PmS by intensifying the incident P waves where caustics form in the gradient structure. This effect may account for the close association of the distant PmS observations with the near PmP caustic in the ROSE data. (PmP designates P-to-P reflection by the Moho.) Figure 4 also shows that a melt body is highly reflective to P waves at short ranges, and these reflections should be quite prominent as bright spots on the Moho in multichannel seismic (MCS) data.

The ranges at which PmS phases are seen in the MAGMA data set on lines 7 and 8 would be consistent with true PmS. The intervening low-velocity ridge structure, however, almost certainly forces the PmS ray paths to be transmitted up through the Moho instead of being reflected. In the most likely case, that the P-to-S conversion occurs on transmission, only the melt-layer model is acceptable.

It is very unlikely that heterogeneities elsewhere in the crust are responsible for the converted shear waves. A near-sea-floor heterogeneity (perhaps a highly altered fault zone) might be placed to scatter nearly horizontally travelling shear waves with about the right delay after the P arrival. These shear waves would show up, however, on the vertical components prominently, contrary to the observations, and they would probably appear at almost all ranges. A low-rigidity dyke structure at the base of the crust would be oriented incorrectly with respect to

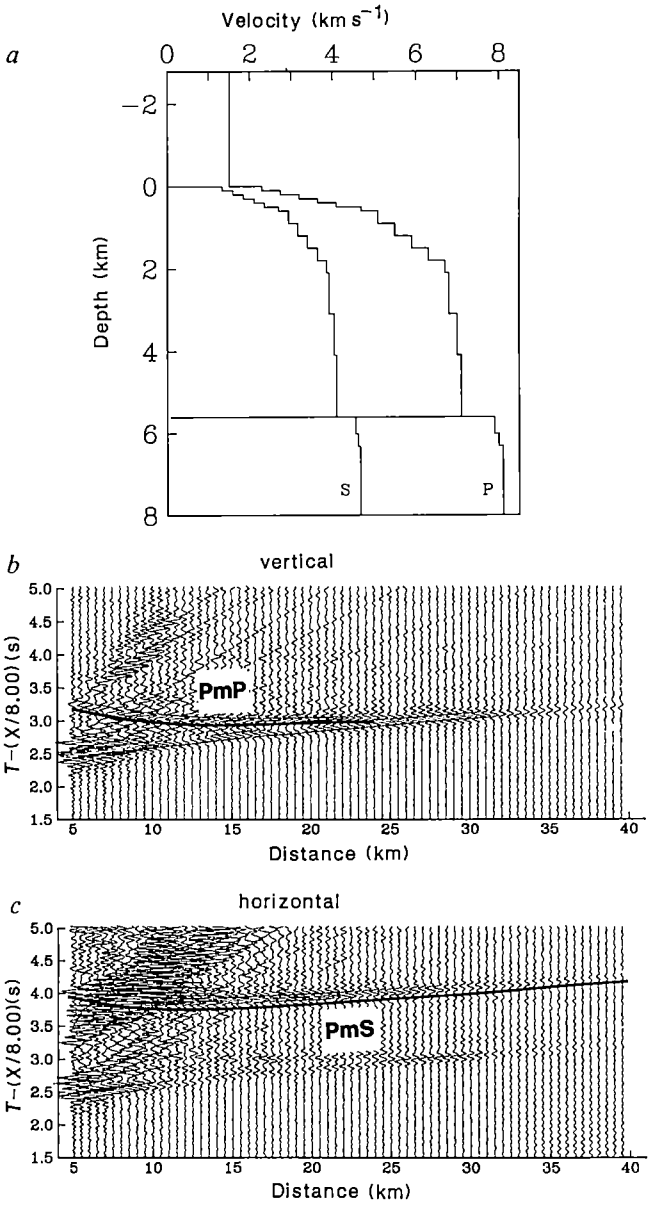

FIG. 4 *a*, The model and *b*, *c*, synthetic seismograms for melt layer at Moho. Water depth 2.8 km. PmS now extends beyond 20 km, mostly diffracted along top of melt layer. Note prominent precritical PmP reflection.

the incident P waves to generate high-amplitude converted shear waves at the observed ranges and times.

It is improbable that Moho topography would be varied enough to intensify PmS sufficiently. The required increase in amplitude is, conservatively, a factor of ten beyond the 20-km range. I assume that this increase requires an expansion of the shear-wave Fresnel-zone area at the Moho (for S-wave propagation from the OBS to the Moho) by roughly the same factor. This requirement results in Moho topographic changes of at least 2-km height over distances of the order of 5 km using the most optimistic assumptions for this mechanism of intensifying PmS. MCS surveys of crustal thickness do not show such large changes over distances this sort[8,9] except at fracture zones[10]. Moho geometries that are less than optimal for this mechanism must cause implausibly abrupt variations in crustal thickness, which would be evident in the seismic data. I conclude that the shear waves are generated by a relatively horizontal body of low rigidity at the Moho, almost certainly a melt-filled sill.

Freezing of the melt bodies at the base of the crust may give rise to a time-varying seismic structure. Repeated OBS or MCS surveys should show these changes if the time scales involved are not too great. For a 10-m-thick sill, the freezing is on the order of a year[11]. MCS surveys may be more useful in providing extensive surveys for the distribution of melt bodies, but they should be supplemented by OBS data for certain identification of melt. There should be unusual range-dependent variations, however, in the P-to-P reflectivity of the anomalous Moho for sufficiently large source-receiver separations. If these variations are discernible, they may provide a useful diagnostic for the presence of melt.

The observation of sills as far as 22 km from the ridge is consistent with a diapir-and-dyke model of magma transport to the crust over broad zones of upwelling and melting, in agreement with recent geodynamical studies[12]. It is reasonable that the most significant compositional boundary in the crystalline oceanic lithosphere would deflect ascending melt. The deepest crustal material close to the ridge is too hot and ductile to permit the melt to ascend by fracturing, so that the rheological and density contrasts at the Moho force the motion of the melt to be close to horizontal. Trapped melt may freeze in place, possibly creating coarse igneous layering in the lower crust. Geological studies of ophiolites[13], which are thought to be slices of oceanic crust emplaced on land, show prominent igneous layering in sections corresponding to the lower crust[13]. Further from the ridge, the crust may become sufficiently cold and brittle that part of the melt escapes by dyke formation (fracture) to the sea floor, forming an off-axis seamount. The seamount at 12° 50′ N is ~5 km from the ridge and could have formed in this way. Melt viscosity and Moho topography are the predominant influences on migration of melt at the Moho, and with sufficient overpressure, melt transport distances and velocities may be large[14]. Thermal expansion of the mantle causes the Moho boundary to slope downwards away from the ridge to provide a mechanical funnel for melt incident on the Moho. It is very likely that a nearly horizontally oriented plumbing system guides melt near the end of its ascent to collect in the magma chambers observed[15] at the ridge. □

Received 17 February; accepted 11 July 1989.

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ACKNOWLEDGEMENTS. I thank J. Orcutt and M. Burnett for supplying me with a copy of the MAGMA data set. I also thank S. Bratt for sending me the lines of MIT OBS data and D. Sandwell, K. MacKenzie, E. Rosencrantz, M. Burnett, A. Harding and R. Batiza for discussions. This work was supported by the NSF.

Modelling the advection of herring larvae in the North Sea

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THE number of fish at the age of first capture in a fishery (recruitment) is dependent on the production of eggs by the parent stock and the survival of early life stages (eggs, larvae and juveniles). In many pelagic fish species the survival of larvae depends on transport from spawning to nursery areas[1]. To investigate larval transport processes for North Sea herring (*Clupea harengus L.*) we have modelled in three dimensions the advection of autumn-spawned larvae during the winter of 1987–1988 and compared the results with sequential field data on the actual distribution of larvae. Circulation in the North Sea is predominantly wind-driven during the winter, and in 1987–1988 anomalous atmospheric conditions caused a reduction in cyclonic circulation and unusual transport of larvae from northern North Sea and west of Scotland spawning areas. Predicting variations in recruitment in advance of fishery legislation has always been difficult and the collapse of North Sea herring populations during the mid-1970s is believed to have been due to a period of several years of low recruitment coupled with high fishing activity[2]. Our results suggest that a better understanding can be achieved with the aid of environmental modelling.

Herring lay eggs on the sea bed off the north and west of Scotland and along the UK coast of the central and northern North Sea, during August and September each year[3–5]. Further spawning takes place in the Southern Bight and English Channel between November and January[3,6]. The larvae hatch within 7–14 days of egg deposition and develop within the plankton until April–June, when they metamorphose into juvenile (0-group) fish[7]. The literature on the winter distribution of herring larvae in the North Sea indicates that larvae from many spawning sites are carried eastward by the water currents towards the continental coast, although some remain in the western North Sea[8–11]. The abundance of autumn-spawned larvae in the southwestern North Sea during the International Young Fish Survey (IYFS) (ref. 12) in February has been correlated with recruitment to the North Sea adult populations during the following years. The absence of larvae in some years has been linked with anomalous circulation of the North Sea[9].

Between August 1987 and March 1988, nine laboratories from Denmark, the Federal Republic of Germany, Norway and the UK participated in the Autumn Circulation Experiment (ACE) (ref. 13), an oceanographic and biological study in the North Sea and off the west of Scotland. One objective of ACE was to examine the distribution of larvae in relation to the water circulation. Field observations of larval distribution between September

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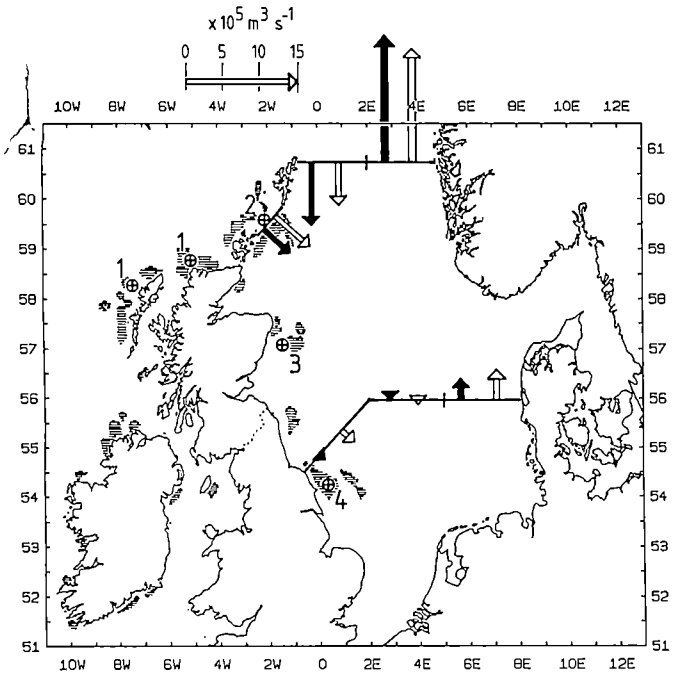


FIG. 1 Geographical distribution of autumn (August–October) spawning areas for herring in the North Sea and west of UK (hatched areas). Numbers refer to starting points for tracers introduced into the three-dimensional advection model: 1) northwest Scotland; 2) Orkney Isles; 3) northeast Scotland; and 4) Yorkshire coast. Open arrows indicate the long-term mean (1969–1982) volume transport during September–December through sections in the North Sea; closed arrows indicate mean volume transport during September–December 1987, calculated from the three-dimensional circulation model.

1987 and March 1988 were compared with distributions predicted by a three-dimensional advection model.

A system comprising two models simulated the advection of herring larvae during ACE (ref. 14). First, a three-dimensional nonlinear numerical model simulated the circulation of the North Sea and adjacent shelf areas[15]. The horizontal grid size of the model was 20 km and the time step was 40 min. The circulation model was forced by the M2 tide, climatological mean summer (May–October) and winter (November–April) stratification, and three hourly surface wind stress and air pressure fields. Daily values of current velocity and variance were calculated by integrating over two tidal cycles. Secondly, the daily three-dimensional current velocity and variance field served as the input to an advection model. The latter model incorporated horizontal and vertical advection, as well as diffusion which was parameterized using the daily current variance. Herring larvae were represented in this model by tracer particles which were programmed to undertake diurnal vertical migrations.

The simulations of herring larvae transport began in September 1987 and continued until the end of February 1988. Four herring spawning areas (starting points for the tracers) were considered (Fig. 1). Tracers were introduced into the model over periods of 10 days. This simulated hatching commenced on 3 October off the Yorkshire coast and on 3 September in all other areas, in accordance with the field estimates of mean hatching dates obtained from the International Herring Larvae Surveys (IHLS) (ref. 16). The total number of tracers introduced in each area was weighted according to the estimates of total production obtained from the IHLS in 1987 (ref. 17).

The vertical position of tracers in the water column can influence their horizontal advection[14]. An active vertical component was therefore imposed on the tracer trajectories to simulate the vertical migrations of herring larvae. In general, larvae

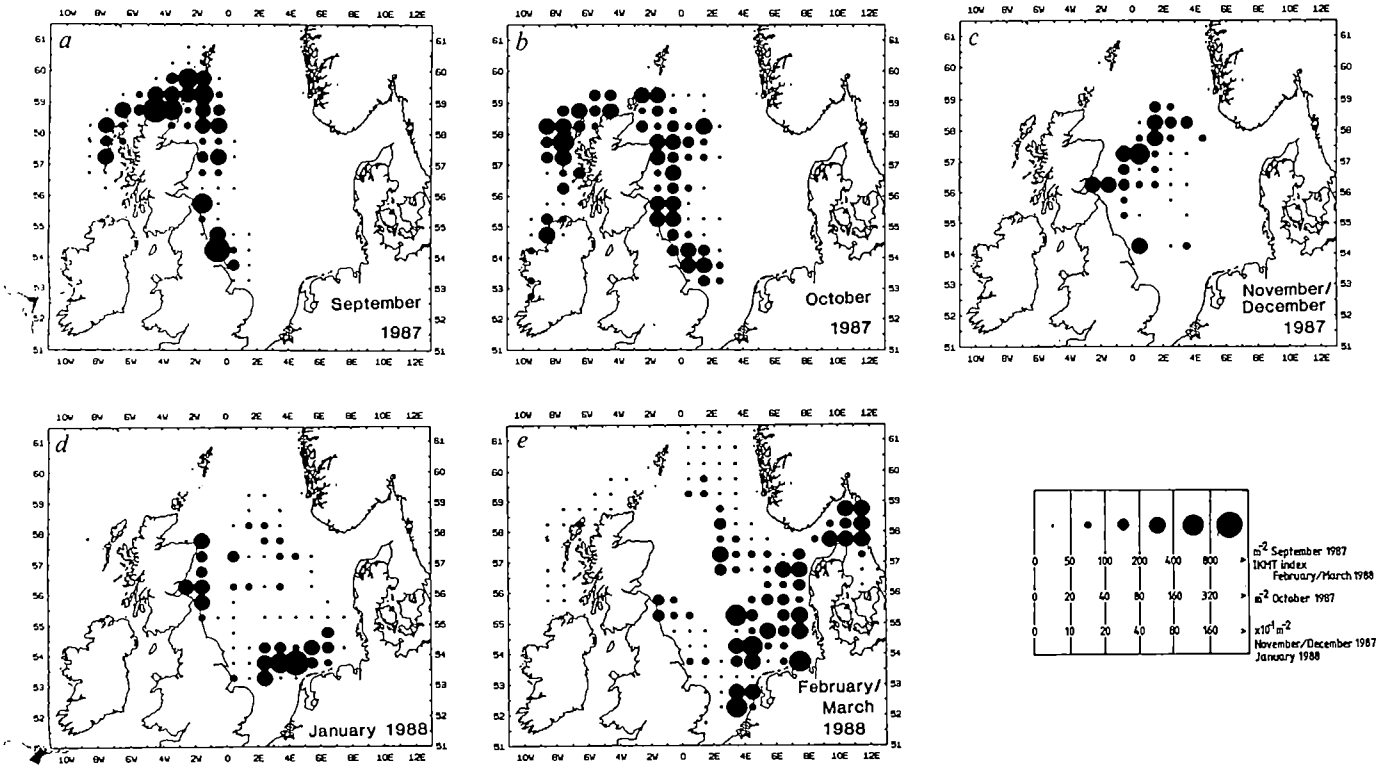
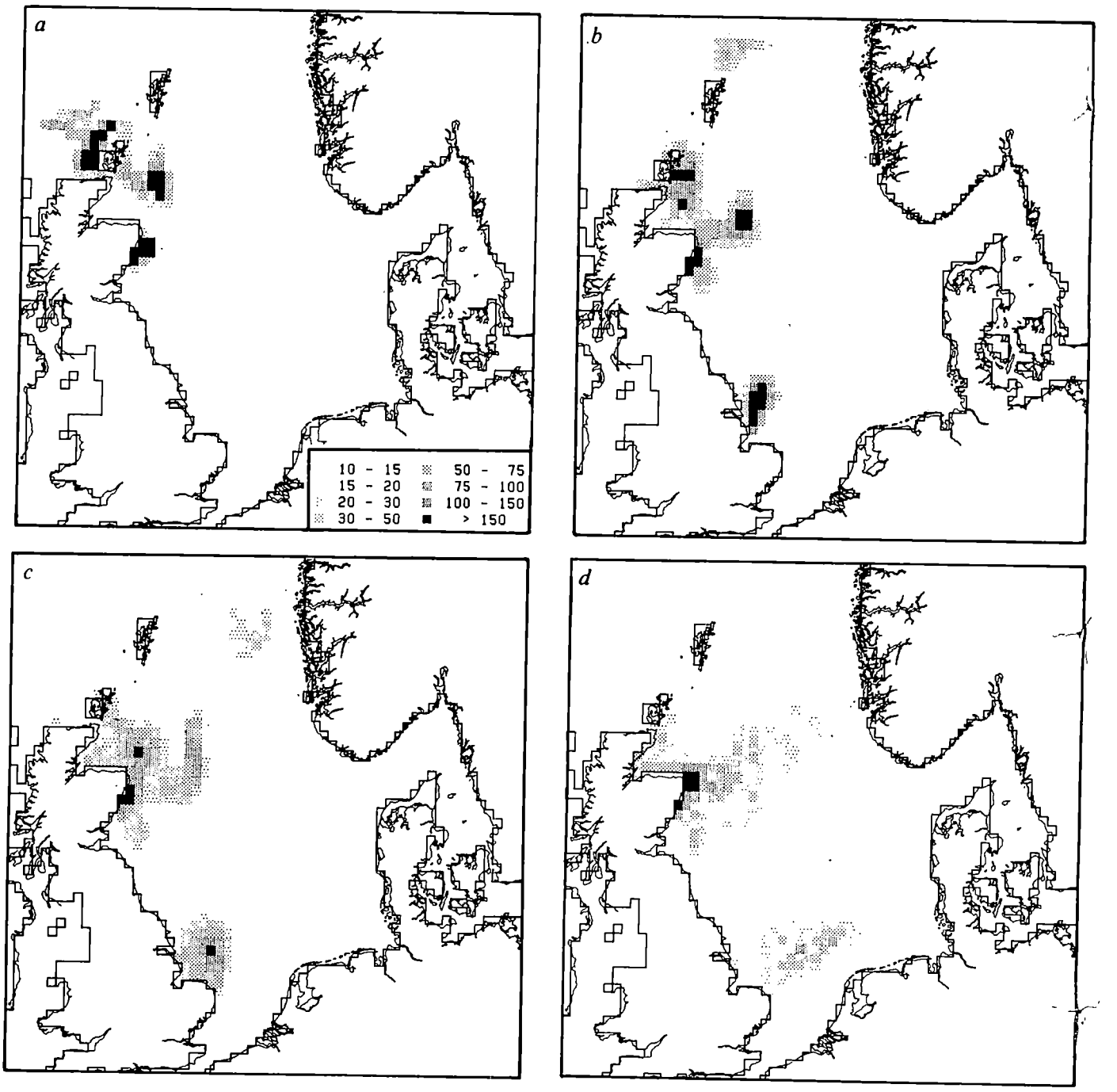


FIG. 2 Field data on the depth-integrated distributions of herring larvae. Data have been averaged over 30′ latitude × 60′ longitude rectangles for presentation. *a*, 1–30 September 1987; *b*, 1–30 October 1987; *c*, 20 November–10 December 1987; *d*, 2–27 January 1988; and *e*, 1 February–16 March 1988. Larvae sampled with: *a* and *b*, a Gulf III (0.03 m^2 mouth, 250 μm aperture mesh)[23], some data from IHLS data base[24]; *c* and *d*, a Methot-Isaacs-Kidd trawl (5 m^2 mouth, 1.5 mm aperture mesh)[25,26]; *e*, an Isaacs-Kidd trawl (10 m^2 mouth, 2.5–5 mm aperture mesh)[27,28], North Sea data from IYFS data base[29]. The northwestern North Sea was not sampled in February 1988 because of incorrect rigging of gear.



undertake diurnal vertical migrations, moving towards the surface during daylight hours and becoming deeper and more dispersed at night (refs 18 and 19; P.M. *et al.*, unpublished results). In the absence of a comprehensive theoretical model of larval migrations, a simplified scheme was used in the advection model: in water deeper than 40 m, the lower level of migration was stipulated to be at 0.6 times the water depth, with the upper level lying 20 m higher. At dusk, the tracers were moved to the lower level over a period of 6 h and remained there until dawn, whereupon they were moved to the upper level, again over 6 h. In water shallower than 40 m, the upper level of migration was fixed at 4 m and the lower level remained the same as for deeper waters. At every time step, each tracer was displaced by the current velocity vector at the actual depth of the tracer.

The atmospheric air pressure and wind stress fields which are the main driving forces of the North Sea circulation during winter showed remarkable deviations from the climatological means during October, November and December 1987 (J. O. Backhaus *et al.*, unpublished data). Strong southerly and southeasterly winds, lasting for periods of days to weeks, alternated with the more usual westerly airflows. This unusual wind field reduced the cyclonic circulation in the North Sea. Results from the circulation model indicated that between September and December, water mass transport into the northwestern North Sea between the Orkney and Shetland Isles was approximately 75% of the long term (1969–1982) mean inflow (D. Hainbucher, *et al.* unpublished data) (Fig. 1), whereas inflow at the western edge of the Norwegian Trench between Shetland and Norway increased to ~140% of the long term mean over the same period. During this period, the influx of northern North Sea water into the southern North Sea was reduced by approximately 50% (compared with the long-term mean), and hence the flushing of the southern North Sea during autumn 1987 was anomalously low.

The spatial distributions of tracers (two-dimensional depth

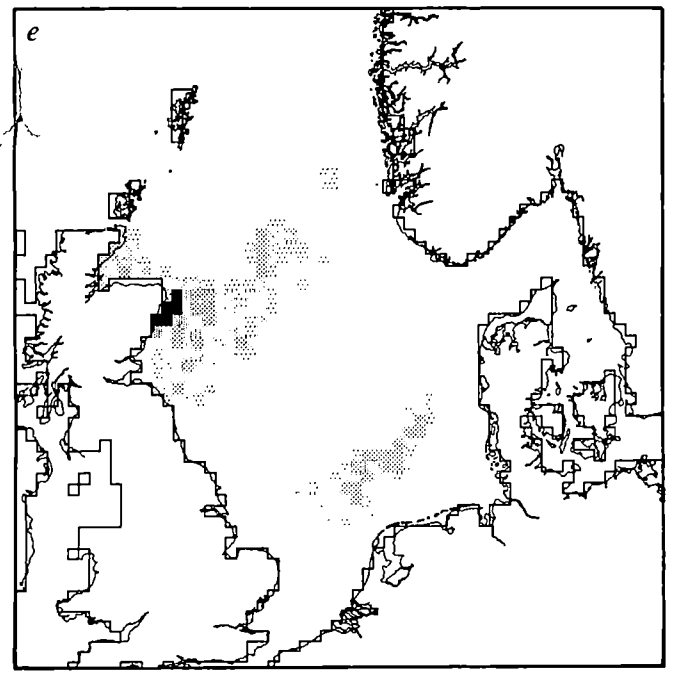


FIG. 3 Two-dimensional (depth-integrated) distribution of tracers from all spawning sites at various dates after introduction into the advection model. a, 30 September 1987; b, 30 October 1987; c, 4 December 1987; d, 28 January 1988; and e, 28 February 1988.

integrated) were taken from the model at 10-day intervals during September and October and at four-day intervals between November and February for comparison with the field study data on herring larvae. No attempt was made to include mortality into the tracer model, so that absolute abundances should not be compared with the field data on the concentration of herring larvae. A comparison, however, of relative distributions of larvae (Fig. 2) and tracers (Fig. 3) is valid. Common features in both the modelled and observed distributions were the persistence of tracers and larvae off the east coast of Scotland throughout the winter, the lack of southerly spreading into the central North Sea, and the eastwards spreading of larvae and tracers from the Yorkshire coast into the German Bight during January and February.

There were two main discrepancies between the modelled and observed distributions of larvae in the central North Sea. First, the rate at which larvae spread through the German Bight and along the Danish coast was more rapid than estimated by the model. Secondly, larvae were present in the inner Skagerrak during surveys in February but no tracers reached the Skagerrak in the simulation. Regular sampling across the mouth of the Skagerrak, along a line running from southern Norway to northern Denmark, indicated that significant numbers of 28–33 mm larvae first arrived in this area in late January[20].

Three observations lead us to believe that most of the larvae arriving in the Skagerrak probably originated from the central North Sea spawning areas. First, local spawning in the Skagerrak is insufficient to account for the concentrations of larvae found in February. Secondly, the mean length of the larvae caught in the Skagerrak in February (30–35 mm) was greater than that of larvae in the northern North Sea (23–26 mm), but similar to that of the larvae found in the southern North Sea in January (28–33 mm). Finally, larvae in the northern North Sea during January and February were associated with water of high salinity ($>35\ g\ kg^{-1}$) and nitrate concentration of approximately 7–8 μM, but water with these characteristics never extended eastwards further than 4 °E, and certainly never entered the Skagerrak. The water in the Skagerrak was characteristically of low salinity ($<33\ g\ kg^{-1}$) and had a concentration of nitrate >12 μM.

Bartsch[14] has shown that the choice of vertical migration model can have a considerable effect on the simulated advection of larvae. An additional simulation of the advection of larvae from the Yorkshire coast spawning site was therefore carried out with a vertical migration pattern in which the larvae were closer to the surface (5 m below the surface during daylight and 15 m at night). The distribution in January with the modified vertical migration pattern was similar to that in the original simulation, but during early February the tracers were rapidly carried northwards along the Danish coast and into the Skagerrak (Fig. 4), roughly corresponding with the field observations of the distibution of larvae. These results suggest that our original scheme for simulating vertical migrations, which was based on observations made in northern waters, was inappropriate in the southern North Sea. Investigations carried out during ACE with a 5-m^2 net and other opening and closing gears did indicate that vertical migrations of larvae followed different patterns in the shallow (<45 m) waters of the southern North Sea from those in the deeper waters of the north.

The eventual fate of larvae in the northern North Sea after February 1988 is not known. The advection model and data on the trajectories of satellite-tracked drifting buoys released during ACE suggest that some may have been carried out of the North Sea in the north-flowing Norwegian coastal current and lost to the North Sea population. Simulations under long-term mean circulation conditions indicate that the northern North Sea larvae would be expected to reach the Skagerrak by February and remain within the North Sea ecosystem[14]. This did not happen in 1987–1988 and we therefore anticipate abnormal recuitment of the 1987 year class to the northern spawning populations in 1990. This expectation is consistent with the conclusion of Bartsch[14] that 'favourable circulation' in the North Sea is a necessary, but not sufficient, prerequisite for good recruitment.

The data from ACE support the use of three-dimensional circulation and advection models for studying the advection and dispersion of vertical migrating fish larvae. Analysis of modelled advection routes over several year classes confirms

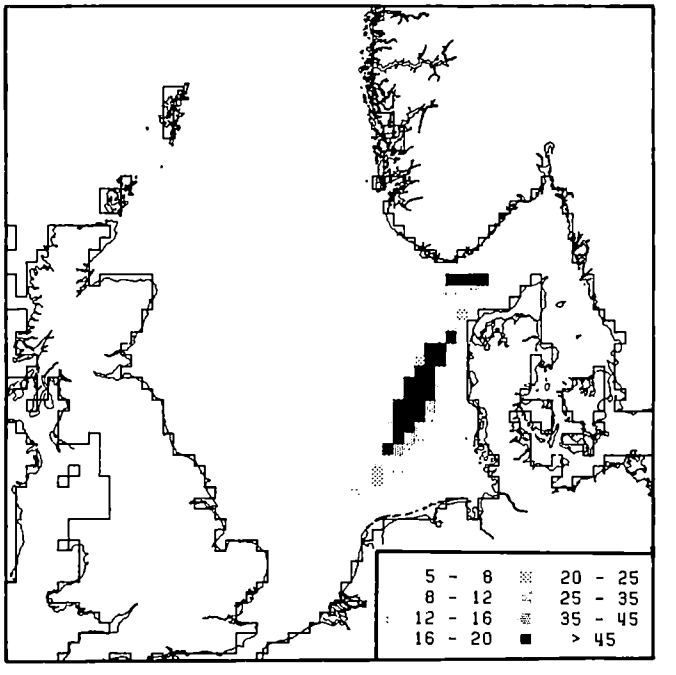


FIG. 4 Two-dimensional (depth-integrated) distribution on 28 February 1988 of tracers from the Yorkshire coast spawning area only, modelled with tracers having a reduced amplitude of vertical migration (5–15 m).

that eastwards dispersion of larvae across the North Sea is the predominant transport phenomenon, but that some larvae invariably remain along the UK east coast[14]. In all years, there is mixing between tracers from widely separated spawning areas. Because the winter circulation in the North Sea is mainly wind-driven[21,22], the pattern of larval dispersion will vary from year to year, depending on the meteorological conditions. □

Received 28 March; accepted 6 July 1989.

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ACKNOWLEDGEMENTS. We thank the captains and crews of the reserach vessels *Challenger, Clione, Dana* and *Scotia* for assisting with our sampling at sea; the International Council for the Exploration of the Sea (ICES) for permission to use Herring Larvae Survey, Herring Assessment and Young Fish Survey data; and our colleagues who participated in ACE. Contribution no. 1 from ACE.

Presynaptic spike broadening reduces junctional potential amplitude

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PRESYNAPTIC modulation of action potential duration may regulate synaptic transmission in both vertebrates[1] and invertebrates[2-4]. Such synaptic plasticity is brought about by modifications to membrane currents at presynaptic release sites, which, in turn, lead to changes in the concentration of cytosolic calcium available for mediating transmitter release. The 'primitive' neuromuscular junction of the jellyfish *Polyorchis penicillatus* is a useful model of presynaptic modulation. In this study, we show that the durations of action potentials in the motor neurons of this jellyfish are negatively correlated with the amplitude of excitatory junctional potentials. We present data from *in vitro* voltage-clamp experiments showing that short duration voltage spikes, which elicit large excitatory junctional potentials *in vivo*, produce larger and briefer calcium currents than do long duration action potentials, which elicit small excitatory junctional potentials.

Synaptic modulation by changes in motor neuron action potential duration serves to synchronize the swimming contractions of the hydrozoan jellyfish *Polyorchis penicillatus*. Action potentials can arise in any depolarized part of the circular, electrically-coupled network of swimming motor neurons[5,6]. As action potentials propagate into progressively more polarized regions of the network, their duration progressively decreases, the excitatory junctional potentials (e.j.ps) elicited in the swimming muscle become larger and the delay to generation of a muscle action potential is reduced[6] (Fig. 1). This decreased delay automatically compensates for the conduction time of the motor spike through the network. These findings are paradoxical, since contemporary models[7] of calcium-mediated release of transmitters predict that postsynaptic potential amplitude should be positively correlated with action potential duration.

As *in vivo* study of membrane ionic currents in the motor neurone network of *Polyorchis* is problematic, because extensive electrical coupling makes voltage-clamp recording ineffective, to further investigate the above phenomena we have developed a method to isolate these cells in primary culture[8]. We have used the whole-cell recording technique to characterize the ionic currents regulating action potential duration and the Ca^{2+} currents which probably mediate transmitter release in the system.

Current-clamp recordings from isolated motor neurons showed that the duration of stimulated action potentials increased with membrane baseline depolarization (unpublished data). We presume this resulted from the steady-state, depolarization-dependent inactivation of an 'A-like', rapidly activating, transient potassium current ($I_{k\text{-fast}}$) which is responsible for action potential repolarization. Voltage-clamp data showed that $I_{k\text{-fast}}$ peaked within 2 to 15 ms, decayed with a time constant of about 200 ms (Fig. 2*a*) and was half-inactivated at a prepulse potential of −47 mV ($N = 4$; Fig. 2*b*). The normal *in vivo* range of resting membrane potentials (−25 mV to

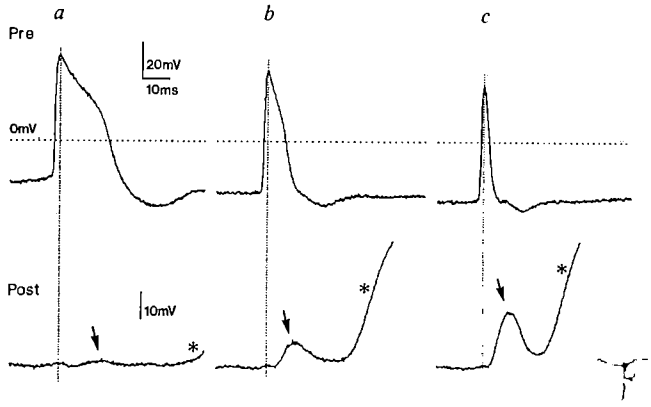

FIG. 1 Motor neuron action potential duration is negatively correlated with muscle e.j.p. amplitude, *in vivo*. These simultaneous pre- and postsynaptic recordings were made across the swimming motor neuron-myoepithelial cell junction in a semi-dissected preparation of the bell margin from *Polyorchis penicillatus* (methods described in ref. 6). Electrodes were positioned ~50 μm apart. Motor neuron action potentials were spontaneously initiated from random sites around the nerve-ring. *a*, When the initiation site was close to the recording electrodes, a broad action potential (upper trace) was seen to arise from a depolarized baseline and elicited a very small e.j.p. (bottom trace, arrow) with a long delay to the muscle action potential (bottom trace, asterisk). *b*, When the initiation site was more distant from the electrodes, the recorded action potential propagated into a more polarized region of the electrically-coupled network, had a shorter duration and elicited a larger e.j.p. (arrow) with a shorter delay to the muscle action potential (asterisk). *c*, An action potential which had propagated from the most distant initiation site to the recording electrodes was seen to arise from the most polarized baseline, had a very short duration and produced the largest e.j.p. (arrow) with the shortest delay to the muscle action potential (asterisk). It is important to note that muscle action potentials occurred at a considerable delay after the e.j.p. since the distance between the recording and muscle spike-initiation sites was large (ref. 6). Electrodes were filled with 2 M potassium acetate. Bath solution was natural sea water at 20 °C. Data were stored on FM tape, then digitized at 200 μs intervals for plotting.

−50 mV) was within the steeper portion of the steady-state inactivation curve for this current, suggesting that $I_{k\text{-fast}}$ was modulated at resting potentials where spike duration varied. A slower outward current, $I_{k\text{-slow}}$, was revealed at more depolarized holding potentials (≥ -30 mV; Fig. 2*a*), where $I_{k\text{-fast}}$ was almost completely inactivated. This slow current activated within 200 ms and decayed *e*-fold in 1–2 s. Tetraethylammonium at 100 mM in the electrode completely blocked both currents. Externally applied 4-aminopyridine at 5 to 10 mM reduced $I_{k\text{-fast}}$ by about 40% after 30 min ($N=8$), while $I_{k\text{-slow}}$ was largely unaffected by this treatment ($N=3$). Both these compounds

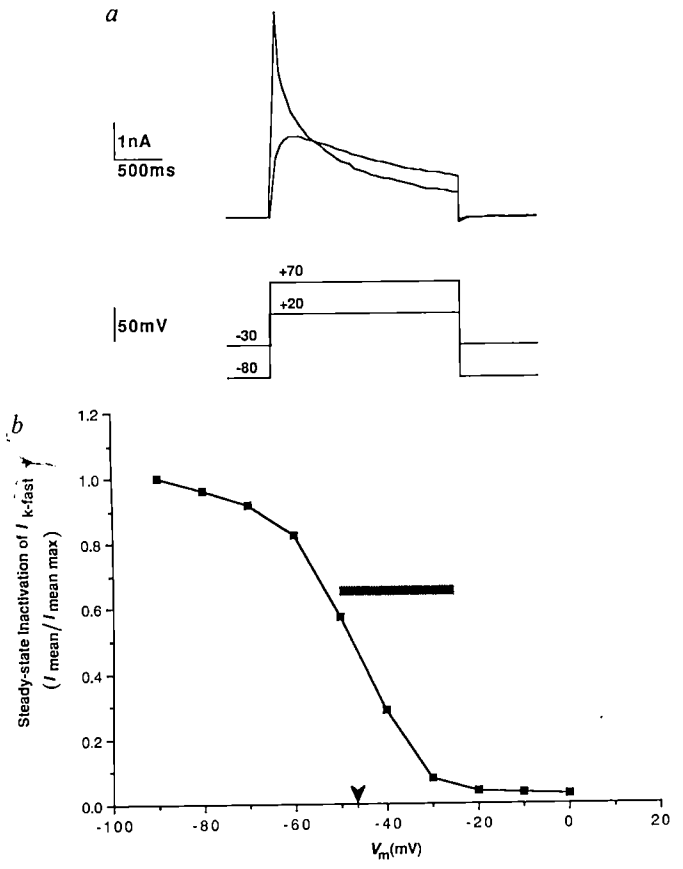

FIG. 2 Potassium currents in isolated motor neurons. *a*, $I_{k\text{-fast}}$ and $I_{k\text{-slow}}$ responses to 100 mV depolarizations applied for 2 s from holding potentials (V_h) of −80 mV and −30 mV, respectively. Current traces were digitized from chart recordings using a 45 ms sampling interval. Voltage protocols were redrawn. *b*, Plot of the voltage-dependence of steady-state inactivation of $I_{k\text{-fast}}$. A 'pre-pulse/pulse' protocol was applied from $V_h=-80$ mV. A 2-s pre-pulse was applied at command voltages incremented in 10 mV steps; a test pulse to +10 mV then followed. Early peak outward currents elicited by the test pulses were measured, leak-subtracted, and normalized to the largest current obtained; these ratios were plotted against pre-pulse potential. This current was active and modulated at *in vivo* resting potentials (shown by stippled bar above plot). Average maximal peak current, elicited after a prepulse potential of −90 mV, was 2.99 nA. Voltage of half-inactivation (−47 mV) is shown by the arrowhead. $N=4$ except for the last three points on the right which are $N=3$, 3 and 2, left to right. Contamination by $I_{k\text{-slow}}$ was assumed to be minimal during the early (<20 ms) phase of the response where measurements were taken, since $I_{k\text{-slow}}$ was only weakly activated at the test potential and arose ten times more slowly than did $I_{k\text{-fast}}$. Sodium-free bath solution was used which contained, in mM: choline-Cl, 437; $MgSO_4$, 5.7; $MgCl_2$, 23.3; $CaCl_2$, 9.5; KCl, 7.8; HEPES, 10; KOH, 5.6. Calcium current was blocked with 0.1–0.5 mM $CdCl_2$ in the bath. Whole-cell recording internal solution contained, in mM: Dextrose, 740; KCl, 105; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; KOH, 35. Experiments were carried out at 20–25 °C. Dissociation and culture techniques are explained[8]. The culture medium in the present experiments had been modified and contained, in mM: NaCl, 378; $MgCl_2$, 29; $CaCl_2$, 9.5; Na_2SO_4, 5.7; KCl, 13.4; choline-Cl, 42; HEPES, 10; NaOH, 5; 50 mg L^{-1} gentamycin sulphate was added. The pH for all soultions was adjusted to 7.5.

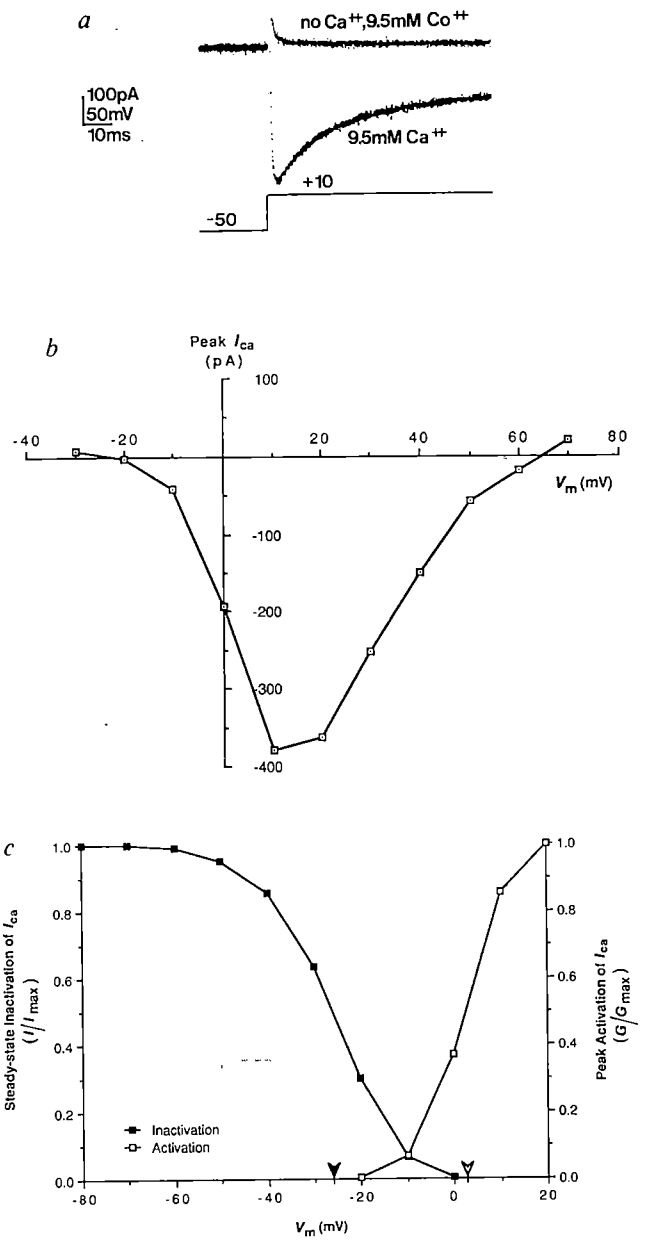

FIG. 3 Calcium current from isolated motor neurones. *a*, I_{Ca} elicited by a 200 ms voltage step from V_h −50 mV to +10 mV. This current was blocked after 5 min in a bathing medium in which Ca^{2+} was replaced by Co^{2+}. *b*, Current/voltage (*I*–*V*) plot for peak I_{Ca}. Depolarizing test-pulses lasting 80 ms were applied from V_h −40 mV to the voltages shown on the abscissa. Leak-corrected responses from 10 cells were averaged for each voltage point, and the peak inward current was plotted. The reversal at +66 mV suggests contamination of I_{Ca} by some residual outward current, evident at more positive V_m. *c*, Steady-state inactivation (solid squares, $N=5$) and peak activation (open squares, $N=10$) of I_{Ca} as a function of V_m. Voltages of half-activation (clear arrowhead, +3 mV) and half-inactivation (solid arrowhead, −26 mV) are indicated. Average maximal peak I_{Ca} for inactivation data was −434 pA; average maximal peak Ca^{2+} conductance for activation data was 7.9 nS. The inactivation data were obtained using a pre-pulse/pulse protocol where V_h was −40 mV, the pre-pulse lasted 1 or 2 and the test pulse went to +10 mV; peak responses were averaged and plotted as a function of pre-pulse potential. The activation curve was drawn from data for *b*; the driving force and conductances were calculated assuming a reversal potential of +66 mV. The range of normal resting potentials is indicated as a stippled bar. Bath solution was the same as in Fig. 2. Internal solution used in *a* and for inactivation data in *c* contained, in mM: CsCl, 140; TEA-Cl, 100; Dextrose, 477; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; NaOH, 28. The *I*–*V* data in *b*, and activation data in *c* were collected using the following, sodium-free electrode solution, in mM: CsCl, 109; TEA-Cl, 100; Dextrose, 536; $MgCl_2$, 2; $CaCl_2$, 1; EGTA, 11; HEPES, 10; CsOH, 31.

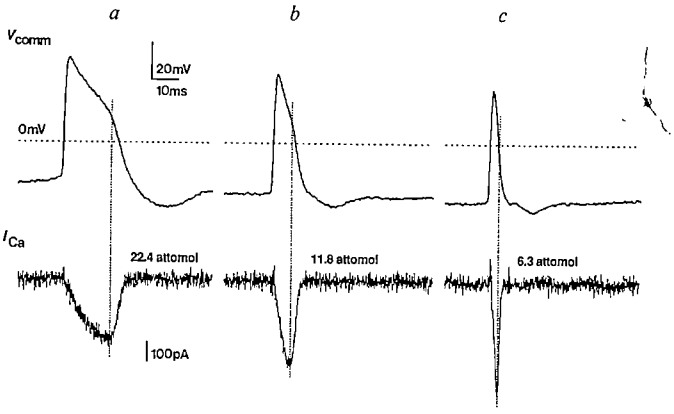

FIG. 4 Calcium currents elicited from *in vitro* motor neurons by spike-shaped command pulses. *a*, A long duration spike command elicited a slowly developing, small amplitude I_{Ca}. Progressively shorter spike commands (*b* and *c*) elicited a progressively more transient and larger ampltiude I_{Ca}. Integrals of these currents are indicated, in attomol (10^{-18} mol) of Ca^{2+} above the current traces. Voltage commands were digitized in 200 μs samples from the FM-recorded action potentials shown in Fig. 1, filtered at 600 Hz to reduce capacitative noise, and applied with 70% series-resistance compensation (final R_{ser} = 1–3 MΩ), through the command input of a List Medical EPC-7 voltage-clamp amplifier. Current responses were digitally recorded on videotape at 44 kHz, then transferred to PCLAMP files at 100 μs per sample for data processing. Capacitance artefacts and leakage were subtracted using responses to inverted, scaled-down (20%) versions of the spikes, applied at the same holding potential as their full-scale counterparts; similar results were obtained if subtraction pulses were applied at more polarized values for V_h. Contaminating, Ca^{2+}-independent currents were subtracted using leakage- and capacitance-corrected responses obtained from the same cell in Ca^{2+}-free, Co^{2+}-substituted saline. All solutions as for Fig. 3*b*. Experiments done at 20 °C.

applied externally caused spike prolongation in current-clamp recordings.

An inward calcium current (I_{Ca}) was recorded from voltage-clamped, cultured motor neurons, which could be eliminated by the replacement of external Ca^{2+} with Co^{2+} (Fig. 3*a*) or by the addition of 0.1–0.5 mM $CdCl_2$. The I_{Ca} activated rapidly, reaching a peak of several hundred picoamperes within 4 ms, and inactivated with a time constant of 15 to 25 ms. In other systems, calcium currents at presynaptic sites[7] normally show slower activation and inactivation. A second time constant of decay (60–70 ms) could be measured suggesting the presence of an additional, smaller and more slowly inactivating component of I_{Ca}. Figures 3*b* and *c* show the voltage-dependence of peak I_{Ca}, peak G_{Ca} activation and peak I_{Ca} steady-state inactivation. It is important to note that these motor neurons have a compact morphology, lacking long or narrow processes[8], and that the synapses they make onto myoepithelial cells are located throughout the neurons[9]. Thus, there is good electrical access to the synaptic sites. We have assumed that the large, transient Ca^{2+} current we observed mediates transmitter release in these motor neurons, although it is possible that a smaller I_{Ca} component may be involved.

To determine the dynamics of voltage-gated calcium influx when action potentials of varying duration invade a presynaptic release site, we stimulated motor neurons *in vitro* using voltage commands shaped like action potentials. Figure 4*a* shows that a long duration action potential elicited a small amplitude, slow calcium current which peaked during the slow repolarizing phase. The same potential *in vivo* had evoked a small amplitude e.j.p. (Fig. 1*c*). In contrast, a short duration action potential produced a larger, more transient calcium current (Fig. 4*c*) and, *in vivo*, had elicited a larger e.j.p. (Fig. 1*c*). Voltage-clamp stimuli of intermediate durations produced calcium currents of intermediate amplitude and time to peak (Fig. 4*b*). Correspondingly, e.j.p. amplitude has been recorded *in vivo* as varying continuously with action potential duration (ref. 6; Fig. 1).

The amplitudes and time courses of calcium currents seen using simulated action potentials can be explained in the following way. The holding potential preceding the spike determines the number of calcium channels avilable for activation and, in part, the peak calcium current elicited by the pulse. Thus, brief spikes arising from a more polarized baseline will be able to activate more calcium channels than prolonged spikes arising from a more depolarized baseline. The rising phase of the spike then activates all available calcium channels (Fig. 4 top; Fig. 3*b*, *c*) but no current flows until a net inward driving force develops, during repolarization. The rapid repolarization of short duration action potentials causes the driving force on Ca^{2+} to increase at a far greater rate than channels are closing and inactivating, resulting in a large, brief calcium current analogous to a 'tail' current. In contrast, the slower rate of repolarization of long duration action potentials is associated with a slower development of the electrical driving force, thus resulting in a more slowly developing calcium current of smaller amplitude.

Measurements of the integrals of I_{Ca} responses reveal that despite the larger peak I_{Ca} elicited by short duration action potentials, long duration action potentials produced a larger total Ca^{2+} influx (Fig. 4). How does a small but rapid entry of calcium release more transmitter, as judged by the amplitude of the e.j.p., than a larger but slower entry of calcium?

There is overwhelming evidence that fast transmitter release depends on the calcium concentration at cytosolic binding sites which regulate the rate of vesicle fusion to the plasma membrane[7,10]. Calcium concentration at these sites, which are thought to be located just beneath the membrane[7,10], is in turn determined by the difference between the rate of calcium influx and the rate of Ca^{2+} removal by diffusion, active pumping, and sequestration[7]. It is possible that the Ca^{2+} sinks in *Polyorchis* motor neurons are very effective and that they counter the increase in submembranous [Ca^{2+}] produced by slow Ca^{2+} influx (during long spikes), while allowing rapid influx to cause a large change in submembranous [Ca^{2+}].

Since these experiments were carried out with 11 mM intracellular EGTA, however, it is possible that Ca^{2+}-dependent inactivation of I_{Ca}, if present, was reduced. Such inactivation would cause the long duration spike to elicit a smaller total calcium influx than observed, which might be less than the influx produced by a short duration spike in native conditions. In this case rapid removal of calcium from cytosolic binding sites would not be necessary to explain the effectiveness of short duration spikes in releasing transmitter material.

This study shows that there can be considerable modulation of the time course of the presynaptic calcium transient by variations in the shape of the presynaptic action potential and that this can lead to differential efficacy of synaptic transmission.

Received 8 May; accepted 18 July 1989.

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ACKNOWLEDGEMENTS. Much of this study was carried out at the Bamfield Marine Station of the Western Canadian Universities Biological Society. We thank Dr G. O. Mackie for comments on the manuscript, Dr N. L. Stockbridge for comments and help in writing the spike waveform generation software. Financial support was provided by an NSERC Operating Grant and CRF Grant (University of Alberta) to A.N.S., and an AHFMR Studentship to JOP.

α-Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating

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IN sympathetic neurons, catecholamines interact with prejunctional α-adrenergic receptors to reduce delivery of transmitter to postjunctional target organs[1–4]. This autoinhibitory feedback is a general phenomenon seen in diverse neurons containing a variety of transmitters[2–4]. The underlying mechanisms of α-adrenergic inhibition are not clear, although decreases in cyclic AMP and cAMP-mediated phosphorylation have been implicated[1–4] (compare ref. 5). We have studied depolarization-induced catecholamine release and calcium-channel currents in frog sympathetic neurons. Here we show that α-adrenergic inhibition of transmitter release can be explained by inhibition of Ca^{2+}-channel currents and not by modulation of intracellular proteins. Noradrenaline strongly reduces the activity of N-type Ca^{2+} channels, the dominant calcium entry pathway triggering sympathetic transmitter release[6], whereas L-type Ca^{2+} channels are not significantly inhibited. The down-modulation of N-type channels involves changes in rapid gating kinetics but not in unitary flux. This is the first detailed description of inhibition of a high-voltage activated neuronal Ca^{2+} channel at the single-channel level. The coupling between α-adrenergic receptors and N-type channels involves a G protein, but not a readily diffusible cytoplasmic messenger or protein kinase C, and may be well suited for rapid and spatially localized feedback-control of transmitter release.

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Figure 1 shows the inhibitory effects of noradrenaline (NA) on transmitter release in isolated frog sympathetic ganglia[7], to facilitate comparisons with measurements of ionic currents in cell bodies (Fig. 2). Noradrenaline strongly and reversibly reduced transmitter outflow evoked by exposure to K^+ (Fig. 1*a*). The inhibition reached a maximum of ~50% at 10–100 μM NA (Fig. 1*b*). Inhibition was unaffected by the β-adrenergic blocker, propranolol, but was prevented by phentolamine (10 μM), a general α-adrenergic antagonist, or by yohimbine (10 μM), an α_2-adrenergic antagonist (Fig. 1*b*). Application of the α_2-adrenergic agonist clonidine (at concentrations ≤100 μM) had no effect. These pharmacological properties are characteristic of a distinct subtype of α_2-adrenergic receptor described in other neurons[8,9]. It has been suggested[1–4] that NA autoinhibition might involve altered phosphorylation of Ca^{2+} channels or of intracellular proteins, such as synapsin I (ref. 10), by cyclic AMP- or Ca^{2+}-dependent protein kinases. We found, however, that the inhibition by 30 μM NA remained unchanged in 1 mM dibutyryl cAMP, or in H-7 (a protein kinase blocker) at a concentration (300 μM) that inhibits several protein kinases[11] (Fig. 1*b*). Transmitter release induced by adding Ca^{2+} in the presence of the Ca^{2+} ionophore ionomycin, bypassing calcium entry through voltage-gated Ca^{2+} channels, was not affected by NA (Fig. 1*b*; ref. 12). This indicates that noradrenergic inhibition does not depend on mechanisms subsequent to a rise in cytosolic Ca^{2+} but is likely to involve modulation of Ca^{2+} entry.

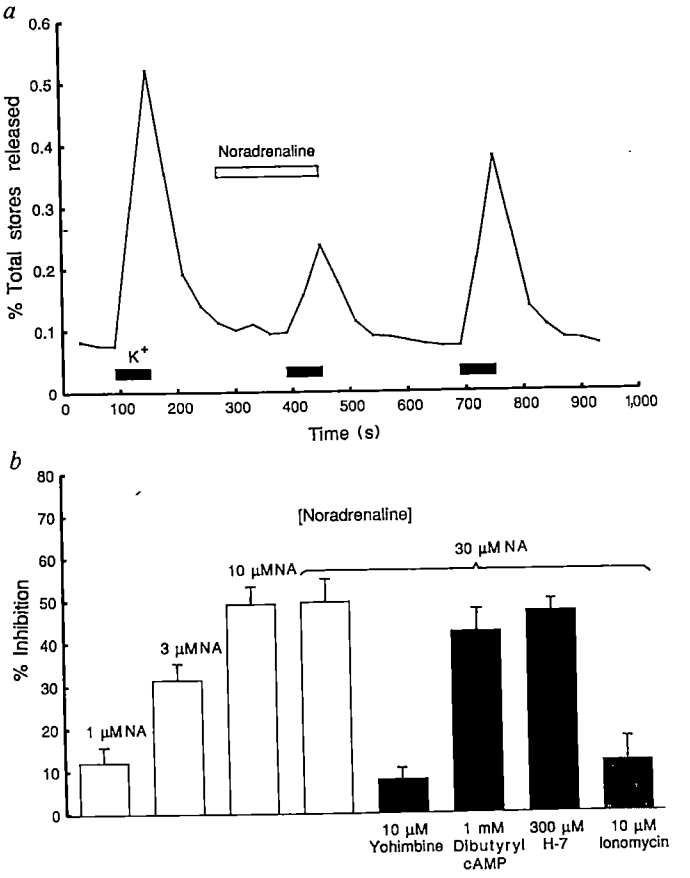

FIG. 1 *a*, Effect of 30 μM NA (open bar) on radiolabelled transmitter release evoked by exposure to 50 mM K^+ (solid bars). *b*, Characteristics of NA effect. Bars represent means ± s.e.m. of percentage of NA-induced inhibition in 3–8 experiments. Open bars, dose-dependence of NA-induced inhibition; solid bars, effect of 30 μM NA in the presence of the indicated agents. Dibutyryl cAMP or H-7 (1-(5-isoquinolinylsulphonyl)-2-methylpiperazine) did not significantly affect NA inhibition ($P > 0.05$). Grey bar, NA did not significantly affect transmitter release evoked by Ca^{2+} in ionomycin ($P > 0.05$). METHODS. Sympathetic chains (3–4 ganglia) from frogs (*Rana pipiens pipiens*) were incubated for 2 h at 22 °C in Ringer's solution containing (mM): NaCl (128), KCl (2), glucose (10), HEPES (10) (*p*H 7.3 with NaOH); also added were ascorbic acid (antioxidant), (1.0 mm) pargyline (monoamine oxidase inhibitor) (0.1 mm), and [^{3}H]-NA (10 μCi ml^{-1} ≈ 0.2 μM) (New England Nuclear). After [^{3}H]-NA loading, the ganglia were enclosed in a chamber and perfused at 1.6 ml min^{-1} with Ringer's solution containing 10 μM desipramine (reuptake blocker), 10 μM propranolol and 2 mM $CaCl_2$. Once a stable baseline of ^{3}H-release was achieved (30–40 min), 30-s fractions were collected continuously. Sympathetic transmitter release (probably a mixture of noradrenaline and adrenaline[36], from neuronal somata[7,27]) was induced by perfusing ganglia with Ringer's solution containing 50 mM K^+ to directly depolarize the cells, bypassing possible changes in action-potential propagation or duration. Release showed dependence on K^+ (30–80 mM) and extracellular calcium (effector concentration for half-maximum response ≈0.5 mM), and complete inhibition by cadmium (50% inhibitory concentration ≈10 μM) as expected for exocytosis triggered by voltage-gated Ca^{2+} influx. The amount of release was calculated as the area (*A*) under each peak after baseline subtraction. The release in the presence of NA (A_{NA}) was expressed as a percentage of the average of release evoked before (A_{con}) and after washing out NA (A_{wash}). Thus, percentage inhibition = $[1-(2A_{NA}/A_{con}+A_{wash})]\times 100$. For ionomycin-induced release, 10 μM ionomycin was added to the perfusion solution (external free calcium concentration buffered at 0.1 μM with 2 mM EGTA) 10 min before collecting fractions. Release was evoked by exposure to 1 mM free calcium. Peak ionomycin-induced release (0.2–0.4%) was similar to that evoked by 50 mM K^+.

Noradenaline inhibits Ca^{2+} entry, as measured by whole-cell Ca^{2+} currents from isolated frog sympathetic neurons (Fig. 2), as it does in other neurons[13–19]. In parallel with the release studies, the inhibition of Ca^{2+} current by NA was antagonized by yohimbine (Fig. 2*b*) and phentolamine, but not by propranolol. Likewise, clonidine (at concentrations up to 100 μM) had no effect. The inhibition was maximal with 10 μM NA (Fig. 2*c*; refs 9, 15). Replacement of GTP in the recording-pipette solution with a non-hydrolysable analogue, GTP-γ-S (0.05 mM), largely prevented recovery from the NA-induced inhibition (Fig. 2*d*); internal GTP-γ-S (0.5 mm) alone mimicked the inhibitory effect of NA (results not shown). Both results suggest involvement of G proteins, as in other neuronal systems[16,20–22].

Sympathetic neurons have two types of high-voltage activated (HVA) Ca^{2+} channels, N-type and L-type, which differ in their voltage and time dependence of inactivation and in their single-channel conductance[6,23–25]. The NA-sensitive current showed partial, but not complete, decay over hundreds of milliseconds[6,25–27] and a strong dependence on holding potential (Fig. 2*e–g*), all consistent with inhibition of N-type Ca^{2+} channels.

Unitary current recordings provide the most direct approach to identifying the NA-sensitive Ca^{2+} channel and characterizing the modulatory effect. Although many neurotransmitters inhibit HVA Ca^{2+} currents[8,9,14–22,24,26], little is known about the mechanism of inhibition at the single-channel level (see ref. 28). Figure 3*a–c* shows N-type and L-type Ca^{2+} channels studied in isolation in the same patch-recording using appropriate voltage protocols[6,23,24]. The N-type channel current illustrated in Fig. 3*a* is sustained during the depolarizing pulse, but in other recordings it often shows a more pronounced time-dependent inactivation[6,24–27]. In all cases, however, the N-type channel current inactivates following prolonged (10–20 s) depolarizations of the holding potential[24,27]. To assess the effect of NA, a series of cell-attached patch recordings were obtained with or without NA (10–100 μM) in the recording pipette. The average N-type Ca^{2+} channel currents were reduced from 0.24 ± 0.09 pA ($n = 22$) to 0.09 ± 0.05 pA ($n = 10$) in 30 μM NA, and to 0.03 ± 0.02 pA ($n = 5$) in 100 μM NA (Fig. 3*f–h*). The L-type Ca^{2+} current was not significantly changed ($P > 0.05$), although there was some hint of inhibition at 100 μM NA. Noradrenaline did not significantly change unitary current amplitude at any concentration tested (legend to Fig. 4). By contrast, there was a marked change in rapid gating kinetics. The mean open time decreased from 0.87 ± 0.14 ms (control; $n = 23$) to 0.38 ± 0.07 ms in 30 μM NA ($n = 7$), or 0.40 ± 0.09 ms in 100 μM NA ($n = 5$). This more than two-fold abbreviation of N-type channel openings contributes substantially to the large decrease in average current seen overall (Fig. 3*f–h*). Changes in gating kinetics on a time scale slower than the pulse duration are suggested by an increase in the percentage of blank sweeps from 8% in the control to 24% in 30–100 μM NA.

The coupling between the α-adrenoceptor and N-type channel inhibition does not involve a readily diffusible second messenger, as application of 100 μM NA to the bath had no effect on N-type channels under the patch pipette (Fig. 3*i, j*; see also ref. 17). In particular, the messenger is unlikely to be cAMP, because dibutyryl cAMP had no effect on N-type channels, whereas it did increase the activity of L-type channels. Protein kinase C is also unlikely to be involved, because phorbol esters increase N-type and L-type Ca^{2+}-channel activity in these cells[29].

Our results demonstrate that NA acts through α-adrenoceptors to selectively inhibit the activity of N-type Ca^{2+} channels, and link this inhibition to attenuation of sympathetic transmitter release. Noradrenaline reduces the probability of channel opening by accelerating the rate of channel closing, and possibly slowing the kinetics of opening, without changing unitary channel flux. Because the mechanism of inhibitory modulation of unitary HVA neuronal Ca^{2+}-channel currents has not previously

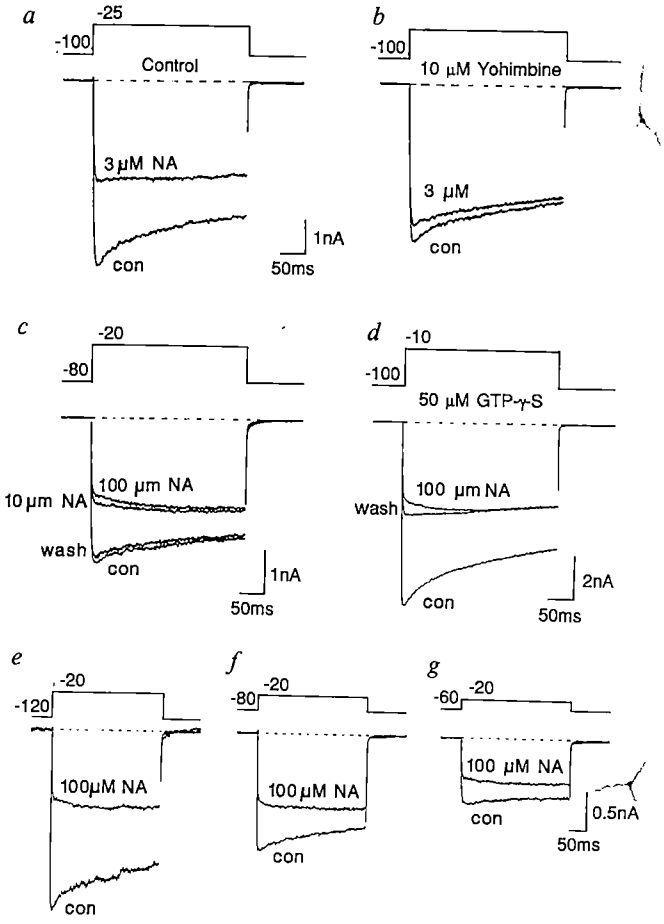

FIG. 2 Noradrenaline inhibition of whole-cell Ca^{2+}-channel currents in sympathetic neurons. *a, b*, Effect of 3 μM NA in the absence and presence of 10 μM yohimbine. Yohimbine block was reversible (data not shown). *c*, Incomplete inhibition of Ca^{2+}-channel currents at maximally effective NA concentrations. Four records were taken: before (con) and after exposure to 10 μM NA, ~30 s after NA removal (wash), and after exposure to 100 μM NA; 10 μM and 100 μM NA were equally effective. Note reversibility of NA effect with standard internal solution (300 μM GTP). *d*, With 50 μM GTP-γ-S instead of internal GTP, the inhibitory effects of NA were irreversible over 9 min of washing in drug-free solution. *e–g*, Recordings of Ca^{2+}-channel currents evoked by step depolarizations to −20 mV from different holding potentials in the absence and presence of NA (100 μM). Same cell as in *c*, 2 mM external Ba^{2+}. The dependence of the NA effect on holding potential is consistent with inhibition of N-type Ca^{2+}-channel current. Under these recording conditions, L-type channels contribute a relatively sustained current, whereas N-type channels show a greater but not complete degree of inactivation with depolarization[6,25–27]. In other experiments with 2–10 mM external Ca^{2+}, NA had little or no effect on sustained currents (presumably L-type channel current) evoked by depolarization from −40 or −30 mV (ref. 37).

METHODS. Neurons were isolated from sympathetic ganglia of adult frogs by a combination of enzymatic and mechanical dissociation (see, for example, ref. 24). Currents carried by voltage-gated Ca^{2+} channels were recorded with the whole-cell patch-clamp method. To minimize Ca^{2+}-dependent inactivation and to block residual outward K^+ currents, Ba^{2+} was usually used as the permeant divalent cation but similar results have been obtained with 2–10 mM Ca^{2+}. The standard internal (pipette) solution contained (mM): CsCl (100), EGTA (10), Na-ATP (2), MgCl (5), GTP (0.3), HEPES (40) (*p*H 7.2 with CsOH). Recording pipettes had resistances <1 MΩ. The external bathing solution contained (mM): tetraethylammonium (TEA) (130), CsCl (5), Glucose (10), $BaCl_2$ (2), tetrodotoxin (TTX) (1 μM), propranolol (10 μM) and HEPES (5, *p*H 7.4 with TEA-OH). Cells were continually superfused at 1–2 ml min⁻¹ with NA-free or NA-containing solutions. A computer (PDP 11/23) controlled the command voltage, and digitized and stored the filtered current signals (−3 dB at 1 kHz). Step depolarizations lasting 320 ms were applied every 10–12 s. All current records were leak-subtracted and are shown together with the voltage protocol. All experiments were carried out at room temperature (~22 °C).

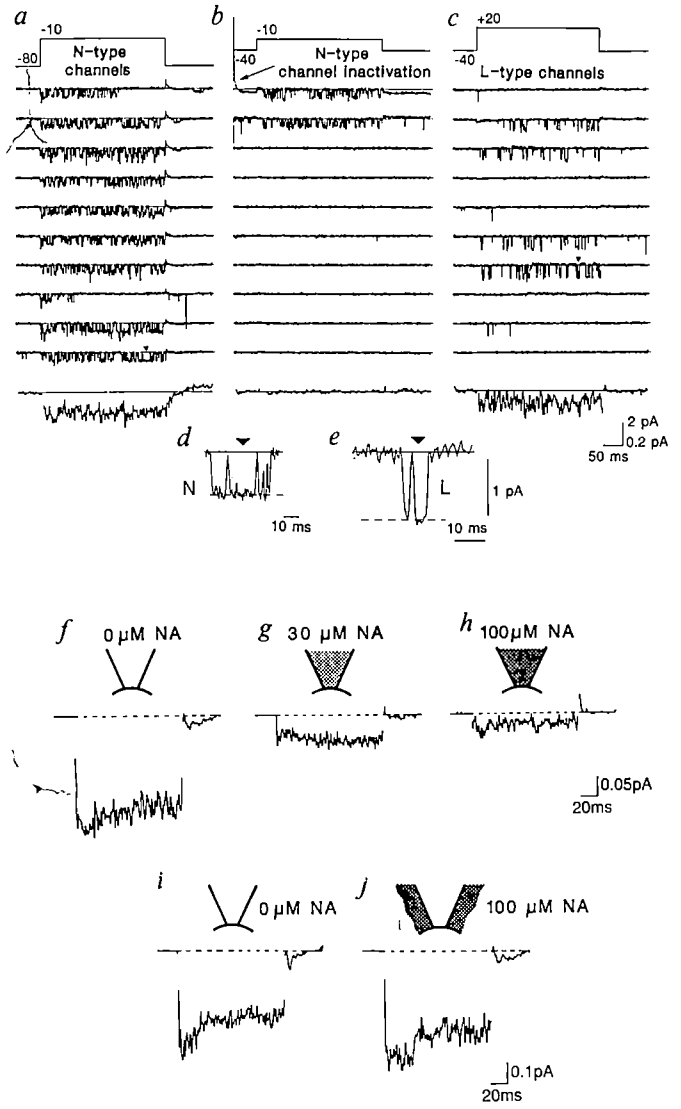

FIG. 3 *a–e*, Separation of unitary N-type and L-type Ca^{2+}-channel currents in the same cell-attached patch. *a*, Sequential recordings of N-type Ca^{2+}-channel currents evoked every 10 s by 320 ms pulses from −80 mV to −10 mV. *b*, Sequential current recordings showing N-type Ca^{2+} channels inactivating within 10–20 seconds following a displacement of the holding potential from −80 to −40 mV (arrow marks corresponding capacitive transient). *c*, Sequential recordings of L-type Ca^{2+}-channel currents evoked by pulses from −40 mV to +20 mV. Average currents, each calculated from ~30 sweeps, are shown below individual current records. *d*, *e* Openings of N- and L-type Ca^{2+} channels (arrow heads in *a*, *c*) enlarged to show clearly resolved and different unitary amplitudes. As the unitary L-type currents at +20 mV (*e*) are larger in amplitude than unitary N-type currents at −10 mV (*d*), despite the smaller driving force for Ba^{2+} entry, they must represent different Ba^{2+} conductances (N-type, 15–16 pS; L-type, 26–28 pS; refs 24, 27). *f–h*, Mean currents from separate groups of cell-attached patches, showing inhibition of N-type Ca^{2+}-channel current evoked by 130 ms pulses from −80 mV to −10 mV. Channel activity was identified as N-type by unitary conductance and sensitivity to holding potential as in *a*, *b*. Averages weighted individual patches equally. *f*, Control ($n=22$ patches); *g*, *h*, 30 μM NA ($n=10$) or 100 μM NA ($n=5$) in the patch pipette. Noradenaline (10 μM) reduced N-type channel currents only slightly; a difference in NA-sensitivity between recordings with 110 mM external Ba^{2+}(*f–h*) and 2 mM Ba^{2+} (Fig. 2) might be expected from alterations in surface potential and local catecholamine concentration. *i*, *j*, Evidence against involvement of a readily diffusible messenger. In five cell-attached patches (pipettes ~1 μm in diameter), the mean current through N-type channels remained unchanged following application of 100 μM NA to the rest of the cell. In none of the experiments was there a detectable decrease in activity after the drug addition.

METHODS. Unitary currents recorded in a series of cell-attached patches with and without NA present in the patch pipette. Attempts at perfusing the pipette with drug while continuously monitoring channel activity were hampered by instability of the recordings, and outside-out patches gave inconsistent results. Recording pipettes had resistances of 5–10 MΩ and contained (mM): $BaCl_2$ (110), HEPES (10), (*p*H adjusted to 7.4 with TEA-OH) and TTX (1 μM). The external bathing solution, used to set the membrane potential to zero, contained (mM): K-aspartate (140), Glucose (10), HEPES (5), EGTA (10) (*p*H adjusted to 7.4 with KOH). A computer (PDP 11/23) controlled the command voltage; and digitized and stored the filtered currents (−3 dB at 1 kHz). Individual current recordings were leak-subtracted before determination of average currents.

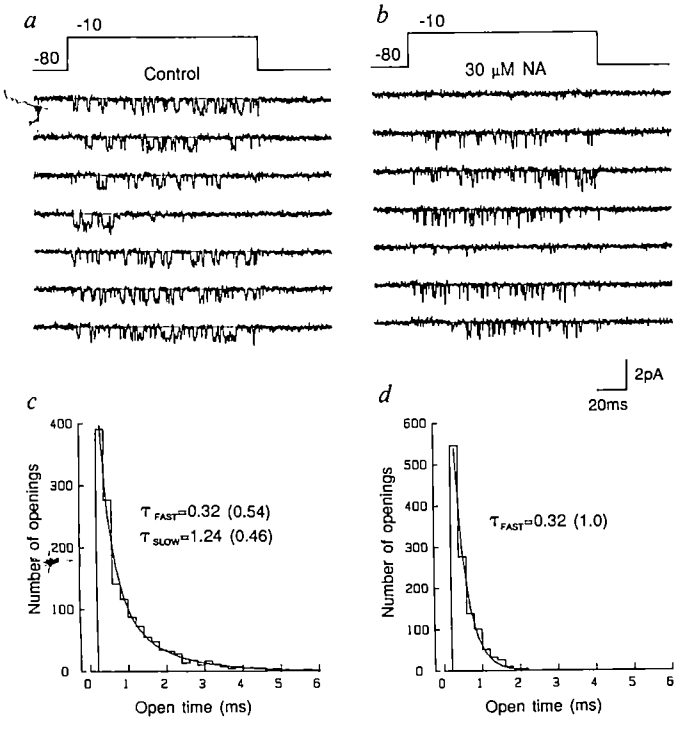

FIG. 4 Effect of NA on the gating of unitary N-type Ca^{2+} channels. *a*, *b*, Sequential sweeps of N-type Ca^{2+} channel currents recorded in two cell-attached patches, in the absence of NA (*a*) and with 30 μM NA present in the pipette solution. (*b*). Noradenaline altered gating kinetics, but did not change unitary current size: control, 0.92 ± 0.02 pA ($n=22$); 30 μM NA, 0.95 ± 0.05 pA ($n=8$); 100 μM NA, 1.0 ± 0.02 pA ($n=5$). *c*, *d*, Histograms of open time durations measured from the patches illustrated in *a*, *b*. Opening and closing events were detected as crossings of a threshold set halfway between open and closed levels. The distribution of open times in the absence of NA (*c*) was fitted by least-squares with the sum of two exponential components of the form $B\exp(-t/\tau)$ with time constants and relative area $[B\cdot\tau/\Sigma(B\cdot\tau)]$ as indicated. In the presence of 30 μM NA, the slow component was essentially absent (*d*), consistent with the diminished appearance of long openings in the current records (*b*). In other experiments, single channel analysis was performed on records with little or no overlap of unitary currents, like those illustrated in *a*, *b*. The slow component was too small to measure in 6 our of 12 patches with 30–100 μM NA. In the other 6 patches, a slow component remained detectable, but it decayed more rapidly and was smaller in amplitude and relative area.

been reported, it will be interesting to see if this pattern of modulation of rapid gating kinetics holds true for other neurotransmitters and other cells[24,26]. Changes in the kinetics of opening and closing might be consistent with allowed voltage-dependence of gating reported by Bean[19].

The pharmacological properties of NA inhibition of whole-cell N-type channel current and transmitter release are very similar. This points to a functional relationship between these phenomena and reinforces earlier evidence that dihydropyridine-insensitive N-type Ca^{2+} channels are the main Ca^{2+} entry mechanism controlling transmitter release from sympathetic neurons[6]. Interestingly, the triggering of transmitter release may be dominated by L-type channels in certain other neuronal systems[30–32]. Stimulation of α-adrenergic receptors is coupled to inhibition of N-type Ca^{2+}-channels and reduction of transmitter release by means of a G protein but not by a readily diffusible second messenger such as cAMP (as in current hypotheses[2–4]), nor by protein kinase C (as in sensory neurons[33]). A relatively direct coupling mechanism would be appropriate for rapid feedback control of transmitter release. The feedback may also be localized, because (1) the concentration of NA falls off steeply with increasing distance from the release sites, (2) the α-adrenergic modulation of N-type Ca^{2+} channels works only at short range, and (3) the attenuation of Ca^{2+} entry may strongly affect only nearby release sites. Our results do not exclude additional effects of NA on potassium channels[34,35], possibly mediated by clonidine-sensitive α_2-receptors[34] and lowered cAMP[35], that would result in less localized decreases in transmitter release through reduction of action-potential duration and global Ca^{2+} entry. □

Received 4 May; accepted 14 July 1989.

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ACKNOWLEDGEMENTS. We thank David Friel, Keith Bley, Rick Aldrich and Dan Madison for suggestions Dr Friel for the use of single channel analysis programs and Dr Aldrich for use of computers. Thi work was supported by the NIH. D.L. is a postdoctoral fellow of the American Heart Association California Affiliate.

A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning

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VOLTAGE-activated potassium channels play an important part in the control of excitability in nerve and muscle. Different K^+ channels are involved in establishing the resting potential, determining the duration of action potentials, modulation of transmitter release, and in rhythmic firing patterns and delayed excitation[1]. Using *in vitro* transcripts made from a directional complementary DNA library we have isolated, by expression cloning in *Xenopus* oocytes, a novel K^+-channel gene (*drk1*). Functionally, *drk1* encodes channels that are K^+ selective and belong to the delayed rectifier class of channels, rather than the A-type class encoded by the *Shaker* gene of *Drosophila*. The channels show sigmoidal voltage-dependent activation and do not inactivate within 500 ms. Structurally, *drk1* encodes an amino-acid sequence which is more closely related to the *Drosophila Shab* gene than to the *Shaker* gene.

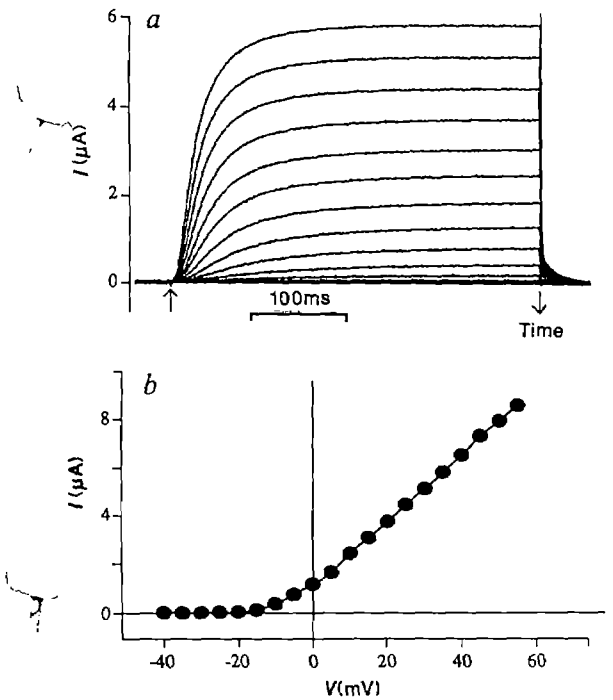
Several cDNA clones encoding K^+ channels have been isolated from *Drosophila*[2–8]. Microinjection of *Xenopus* oocytes, with RNA transcribed *in vitro* from some of the clones produces voltage-dependent, fast transient outward K^+ currents, characteristic of A-type channels[9–11]. The different cDNA clones that have been isolated contain an identical core region, yet differ in the regions encoding the amino and carboxyl termini of the proteins. Using *Shaker* sequence information, two mammalian K^+-channel cDNA clones encoding the same gene were recently isolated by cross-hybridization[12,13]. Expression of rat cDNAs in *Xenopus* oocytes yielded K^+ currents with properties of delayed rectifier channels[14] and possibly A-type channels[15]. We designed a sequence-independent approach to isolate cDNA clones encoding channel and receptor genes expressed in the brain. Size-fractionated rat-brain messenger RNA, enriched for mRNA between 3.3 and 4.2 kilobases (kb), was used to generate a directional cDNA library in the transcription-competent vector λZAP[16]. Pools of 100,000 recombinant phages (independent cloning events) were amplified and used to prepare DNA templates for RNA synthesis. Following microinjection of *in vitro* synthesized RNA, *Xenopus* oocytes were tested for outward currents (I_K) produced by depolarizing voltage steps. Transcripts from pools of 100,000 recombinants yielded small I_K-like currents. One pool was divided into smaller 'cocktails', each containing 10,000 recombinants. Three of seven such cocktails elicited sustained outward currents of several hundred nA in amplitude upon depolarization of the oocyte membrane. The currents activated relatively slowly (>100 ms for full activation) and did not inactivate during the test pulse (500 ms). One cocktail was chosen and, by reducing the pool size to 1,000, then 100 and finally 12 recombinants, we eventually isolated a single 'positive' clone (*drk1*) with a 3.4-kb insert.

The cDNA clone we isolated encoded a K^+ channel with the properties of a delayed rectifier[1]. The channels opened at test potentials more positive than −20 mV and showed sigmoidal voltage-dependent activation (Fig. 1*a*, *b*). The time to half-maximal activation ranged from 20 to 100 ms. Injection of as little as 20 pg *in vitro*-synthesized transcripts produced I_K-current amplitudes of up to 1 μA at +40 mV. At higher current

densities, resulting from injection of 2 ng RNA, it was possible to record from macropatches containing several hundred channels, where the changes in potential are complete in much less than 1 ms (manuscript in preparation). Voltage dependence and kinetics were similar for both macropatch and whole-cell recording. To characterize the ionic selectivity of these channels we determined the reversal potential from tail-current measurements (Fig. 1*c*). Substituting external Na^+ with *N*-methyl-D-glucamine$^+$ (NMDG) had a negligible effect, whereas varying the external K^+ concentration ($[K^+]$) shifted the reversal potential with a slope of 48 mV per 10-fold charge in external $[K^+]$ (Fig. 1*d*), which is characteristic of channels selective for K^+ ions over Na^+ ions. These K^+ currents were blocked by 4-aminopyridine with a 50% inhibitory concentration (IC_{50}) of 0.5 mM and by tetraethylammonium with an IC_{50} of 10 mM (data not shown). The outward current at +20 mV was independent of the presence of Ca^{2+} in the bath and insensitive to Co^{2+} (2 mM) or Cd^{2+} (0.2 mM), eliminating the possibility of the presence of Ca^{2+}-activated K^+ channels. Neither apamin nor charybdotoxin at concentrations up to 1 μM had any effect on the channels.

The DNA sequence of *drk1* was determined and shows an open reading frame of 2,559 nucleotides (Fig. 2*a*) encoding a protein of 853 amino acids (with a calculated relative molecular mass of 95,294). The 5′-untranslated region contains 13 nucleotides (the first 8 nucleotides shown in Fig. 2*a* are linker-derived) and no upstream ATG preceding the assigned initiation codon (that is, the first ATG found). The TGA stop codon is followed by a 3′-untranslated region of ~800 nucleotides ending in a poly(A) tail of ~50 residues. The beginning of the derived protein sequence contains hydrophilic and charged amino acids and presumably does not represent a signal peptide. A hydropathy plot of the deduced amino-acid sequence shows features reminiscent of all voltage-gated ion channels cloned so far (Fig. 2*b*). In the N-terminal half of the molecule, there are six hydrophobic regions that have been attributed to membrane spanning segments (boxed regions Fig. 2*b*). The other half of the protein encoded by *drk1* does not show main hydrophobic regions and probably represents a cytoplasmic tail of >400 amino-acid residues. It is longer than the corresponding C-termini of all other K^+ channels known and it does not show similarity to any known sequence in the protein-sequence data bank. This part of the *drk1* product contains two consensus sequences for cyclic AMP-dependent phosphorylation[17]. The tail may be important in modulation of channel activity and/or targeting of the channel to specific subcellular compartments.

The N-terminal half of the amino-acid sequence derived from the *drk1* clone is similar to the core regions of K^+ channels of



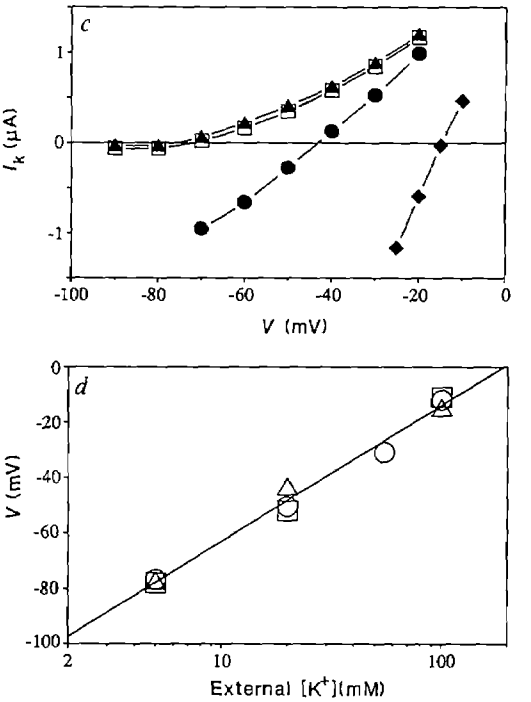


FIG. 1 Delayed rectifying kinetics and K^+ selectivity of *drk1* mRNA expressed in *Xenopus* oocytes. *a*, Family of K^+ currents elicited by 400 ms voltage steps from a holding potential of −50 mV to test potentials ranging from −40 mV to +30 mV in 5-mV increments. The beginning and the end of the pulse are indicated by arrows. Leakage and capacity currents are corrected for by adding the response to a voltage step of equal size but opposite sign. *b*, Steady-state current–voltage relationship for the experiment shown in *a*. The current at the end of the 400-ms test pulse is plotted against membrane potential. Threshold for activation was −15 mV. *c*, The effect of varying external K^+ and Na^+ concentrations on the instantaneous current–voltage relationship. The K^+ conductance was activated by a 200-ms voltage step to 0 mV and repolarized to potentials between −10 mV and −100 mV in 10-mV increments. The instantaneous current following the repolarization is estimated by fitting a double-exponential function to the tail-currents which is extrapolated back to the time of the step. The resulting instantaneous currents are corrected for a linear leakage component estimated from the steady-state currents between −50 and −100 mV and plotted against voltage. The external solutions contained $MgCl_2$ (2 mM). $CoCl_2$ (2 mM), HEPES (10 mM, pH 7.4) to which was added (mM): NaCl (100), KCl (5) (□), NMDG (100), KCl (5) (▲), NMDG (85), KCl (20) (●), NMDG (5), KCl (100) (◆). NMDG was used to replace Na^+. *d*, Reversal potential as a function of external K^+ concentration. Reversal potentials were measured from instantaneous current–voltage relationships as shown in *c*. Results from three oocytes are combined. A linear least-squares fit is shown with a slope of 48 mV per 10-fold change in external $[K^+]$.

METHODS. A directional rat-brain cDNA library enriched for full-length inserts between 3.3–4.2 kb[16] was used for *in vitro* transcription of sense-strand RNA using T7 RNA polymerase and the 5′-cap analogue $m^7G(5')ppp(5')G$ (ref. 19). After microinjection of RNA (5 ng), *Xenopus* oocytes were screened 3–4 days later for the expression of delayed rectifying K^+ channels using a two-electrode voltage-clamp technique (ref. 20, and R.H.J. *et al.*, manuscript in preparation). A 'positive' pool of 100,000 recombinants was subdivided into pools of progressively smaller sizes and eventually led to the isolation of the clone *drk1*. Oocytes were isolated from *Xenopus* frogs as described elsewhere (ref. 20, and R.H.J. *et al.*, manuscript in preparation) and kept in modified Barth's solution at 19 °C. For screening they were transferred to a recording chamber and kept in a solution containing (mM): NaCl (140), KCl (2), $CaCl_2$ (2), $MgCl_2$ (2), HEPES (10, pH 7.4). Electrodes were filled with KCl (2 M), HEPES (10 mM, pH 7.2). Their resistance in the bath ranged from 1 to 3 MΩ. Oocytes were voltage-clamped using an Axoclamp 2A (Axon Instruments).

a

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  1                                                MetThrLysHisGlySerArgSerThrSerSerLeuPro
-21  AATTCCGGTCGCTGGTCTGGCATGACGAAGCATGGCTCGCGCTCCACCAGCTCGCTGCCG

 14  ProGluProMetGluIleValArgSerLysAlaCysSerArgArgValArgLeuAsnVal
 40  CCCGAGCCCATGGAGATCGTGCGCAGCAAGGCGTGCTCGCGCCGGGTGCGCCTCAACGTC

 34  GlyGlyLeuAlaHisGluValLeuTrpArgThrLeuAspArgLeuProArgThrArgLeu
100  GGGGGCCTGGCGCACGAGGTGCTGTGGCGCACTCTGGACCGCCTGCCCCGCACGCGGCTG

 54  GlyLysLeuArgAspCysAsnThrHisAspSerLeuLeuGlnValCysAspAspTyrSer
160  GGCAAGCTTCGGGACTGCAACACGCACGACTCCCTGCTCCAGGTGTGCGACGACTACAGC

 74  LeuGluAspAsnGluTyrPhePheAspArgHisProGlyAlaPheThrSerIleLeuAsn
220  CTTGAGGACAACGAGTACTTCTTCGACCGCCACCCTGGCGCCTTTACCTCCATTCTCAAT

 94  PheTyrArgThrGlyArgLeuHisMetMetGluGluMetCysAlaLeuSerPheSerGln
280  TTCTACCGCACCGGCCGGCTGCACATGATGGAGGAGATGTGCGCGCTGAGCTTCAGCCAG

114  GluLeuAspTyrTrpGlyIleAspGluIleTyrLeuGluSerCysCysGlnAlaArgTyr
340  GAGCTGGACTACTGGGGCATCGATGAGATCTACCTGGAGTCCTGCTGCCAGGCCCGCTAT

134  HisGlnLysLysGluGlnMetAsnGluGluLeuLysArgGluAlaGluThrLeuArgGlu
400  CACCAAAAGAAGGAGCAGATGAACGAGGAGCTGAAGCGCGAGGCCGAGACGCTGCGGGAG

154  ArgGluGlyGluGluPheAspAsnThrCysCysAlaGluLysArgLysLysLeuTrpAsp
460  CGGGAGGGCGAGGAGTTTGACAACACGTGCTGTGCAGAGAAGAGGAAGAAACTCTGGGAT
                                                    S1
174  LeuLeuGluLysProAsnSerSerValAlaAlaLysIleLeuAlaIleIleSerIleMet
520  CTGCTGGAGAAGCCCAACTCGTCGGTGGCCGCCAAGATCCTGGCCATCATCTCGATCATG

194  PheIleValLeuSerThrIleAlaLeuSerLeuAsnThrLeuProGluLeuGlnSerLeu
580  TTCATCGTCCTGTCCACCATCGCTCTGTCACTCAACACGCTGCCTGAGCTGCAGAGCCTA

214  AspGluPheGlyGlnSerThrAspAsnProGlnLeuAlaHisValGluAlaValCysIle
640  GACGAGTTCGGCCAGAGCACGGATAACCCGCAGCTGGCGCACGTGGAGGCGGTGTGCATC
          S2
234  AlaTrpPheThrMetGluTyrLeuLeuArgPheLeuSerSerProLysLysTrpLysPhe
700  GCATGGTTCACCATGGAGTACTTGCTGAGGTTCCTGTCCTCCCCCAAGAAATGGAAGTTC
                                                    S3
254  PheLysGlyProLeuAsnAlaIleAspLeuLeuAlaIleLeuProTyrTyrValThrIle
760  TTCAAGGGCCCCCTCAACGCCATTGACCTACTGGCCATCCTGCCCTACTACGTCACCATC

274  PheLeuThrGluSerAsnLysSerValLeuGlnPheGlnAsnValArgArgValValGln
820  TTCCTCACAGAATCCAACAAGAGCGTGCTGCAGTTCCAGAACGTGCGCCGCGTGGTCCAG
                    S4
294  IlePheArgIleMetArgIleLeuArgIleLeuLysLeuAlaArgHisSerThrGlyLeu
880  ATCTTTCGCATCATGCGCATCCTTCGGATCCTGAAGCTGGCCCGTCACTCCACTGGCCTG

314  GlnSerLeuGlyPheThrLeuArgArgSerTyrAsnGluLeuGlyLeuLeuIleLeuPhe
940  CAGTCCTTGGGCTTCACCCTGCGCAGAAGCTACAACGAGCTGGGCCTGCTCATCCTCTTC
          S5
334  LeuAlaMetGlyIleMetIlePheSerSerLeuValPhePheAlaGluLysAspGluAsp
1000 CTCGCGATGGGCATCATGATCTTCTCCAGCCTGGTCTTCTTCGCGGAGAAGGATGAGGAC

354  AspThrLysPheLysSerIleProAlaSerPheTrpTrpAlaThrIleThrMetThrThr
1060 GACACCAAGTTCAAAAGCATCCCCGCCTCTTTCTGGTGGGCTACCATCACCATGACAACT

374  ValGlyTyrGlyAspIleTyrProLysThrLeuLeuGlyLysIleValGlyGlyLeuCys
1120 GTTGGCTATGGAGACATCTACCCGAAGACTCTCCTGGGGAAAATCGTGGGGGGGCTCTGT
                    S6
394  CysIleAlaGlyValLeuValIleAlaLeuProIleProIleIleValAsnAsnPheSer
1180 TGCATTGCCGGGGTCCTGGTGATTGCTCTCCCCATCCCCATCATCGTCAATAACTTCTCC

414  GluPheTyrLysGluGlnLysArgGlnGluLysAlaIleLysArgArgGluAlaLeuGlu
1240 GAGTTCTACAAGGAGCAGAAGCGCCAGGAGAAAGCCATCAAGCGGAGGGAGGCTCTGGAG

434  ArgAlaLysArgAsnGlySerIleValSerMetAsnMetLysAspAlaPheAlaArgSer
1300 AGAGCCAAGAGGAATGGCAGCATCGTGTCCATGAACATGAAGGACGCCTTCGCCCGGAGC

454  IleGluMetMetAspIleValValGluLysAsnGlyGluSerIleAlaLysLysAspLys
1360 ATCGAGATGATGGACATCGTGGTGGAGAAAAATGGCGAGAGCATAGCTAAGAAGGATAAA

474  ValGlnAspAsnHisLeuSerProAsnLysTrpLysTrpThrLysArgAlaLeuSerGlu
1420 GTGCAAGATAACCACCTGTCCCCCAACAAGTGGAAATGGACCAAGAGGGCACTCTCCGAG

494  ThrSerSerSerLysSerPheGluThrLysGluGlnGlySerProGluLysAlaArgSer
1480 ACCAGCTCCAGTAAGTCCTTTGAAACCAAGGAGCAGGGATCCCCCGAAAAGGCCAGGTCC

514  SerSerSerProGlnHisLeuAsnValGlnGlnLeuGluAspMetTyrSerLysMetAla
1540 TCGTCTAGTCCACAGCACTTGAACGTCCAGCAGCTGGAAGACATGTACAGTAAGATGGCC

534  LysThrGlnSerGlnProIleLeuAsnThrLysGluMetAlaProGlnSerLysProPro
1600 AAGACACAGTCCCAGCCCATCCTCAACACCAAGGAGATGGCCCCGCAGAGCAAGCCTCCA

554  GluGluLeuGluMetSerSerMetProSerProValAlaProLeuProAlaArgThrGlu
1660 GAGGAGCTGGAGATGAGCAGCATGCCCAGCCCCGTGGCCCCTCTGCCCGCACGCACGGAG

574  GlyValIleAspMetArgSerMetSerSerIleAspSerPheIleSerCysAlaThrAsp
1720 GGCGTCATCGACATGCGGAGCATGTCCAGCATTGACAGCTTCATCAGCTGTGCCACGGAC

594  PheProGluAlaThrArgPheSerHisSerProLeuAlaSerLeuSerSerLysAlaGly
1780 TTCCCTGAAGCCACCAGATTCTCCCACAGTCCTCTGGCATCCCTCTCCAGCAAGGCTGGG

614  SerSerThrAlaProGluValGlyTrpArgGlyAlaLeuGlyAlaSerGlyGlyArgLeu
1840 AGCAGCACAGCCCCGGAGGTGGGCTGGCGGGGGGCTCTGGGTGCCAGCGGCGGGAGGCTC

634  ThrGluThrAsnProIleProGluThrSerArgSerGlyPhePheValGluSerProArg
1900 ACGGAGACCAACCCCATTCCTGAGACCAGCCGCTCTGGTTTCTTCGTGGAGAGTCCCAGG

654  SerSerMetLysThrAsnAsnProLeuLysLeuArgAlaLeuLysValAsnPheValGlu
1960 AGTTCCATGAAGACCAACAACCCCTTGAAGCTGCGAGCGCTCAAGGTCAACTTCGTGGAG

674  GlyAspProThrProLeuLeuProSerLeuGlyLeuTyrHisAspProLeuArgAsnArg
2020 GGCGACCCCACCCCGCTGCTGCCCTCCCTGGGCTTGTATCACGATCCTCTTAGGAACAGA

694  GlyGlyAlaAlaAlaAlaValAlaGlyLeuGluCysAlaSerLeuLeuAspLysProVal
2080 GGAGGGGCAGCGGCTGCAGTTGCGGGACTGGAGTGTGCCTCCCTCTTAGACAAGCCCGTG

714  LeuSerProGluSerSerIleTyrThrThrAlaSerAlaArgThrProProArgSerPro
2140 CTGAGCCCAGAGTCCTCCATCTACACCACAGCAAGTGCCAGGACGCCCCCTCGCTCCCCT

734  GluLysHisThrAlaIleAlaPheAsnPheGluAlaGlyValHisHisTyrIleAspThr
2200 GAGAAACACACAGCAATAGCATTCAACTTCGAGGCGGGAGTCCACCATTACATAGACACC

754  AspThrAspAspGluGlyGlnLeuTyrSerValAspSerSerProProLysSerLeu
2260 GACACAGATGACGAGGGTCAGCTGCTCTACAGCGTGGACTCCAGCCCTCCCAAAAGTCTC

774  HisGlySerThrSerProLysPheSerThrGlyAlaArgThrGluLysAsnHisPheGlu
2320 CACGGGAGTACCAGTCCCAAGTTCAGCACTGGAGCTAGAACGGAGAAGAACCACTTCGAG

794  SerSerProLeuProThrSerProLysPheLeuArgProAsnCysValTyrSerSerGlu
2380 AGCTCCCCCTTGCCCACCTCCCCTAAGTTCTTAAGGCCGAACTGTGTCTACTCCTCAGAA

814  GlyLeuThrGlyLysGlyProGlyAlaGlnGluLysCysLysLeuGluAsnHisThrPro
2440 GGGTTGACTGGAAAAGGCCCTGGGGCTCAAGAGAAATGCAAGCTGGAGAACCATACCCCC

834  ProAspValHisMetLeuProGlyGlyGlyAlaHisGlySerThrArgAspGlnSerIle
2500 CCGGATGTGCACATGCTGCCTGGGGGAGGAGCACACGGGAGCACTAGGGATCAGAGTATC

2560 TGAGCTCCCTACCACAGAGGGCATCTGCA-----(approx 800nucleotides)-----3'end
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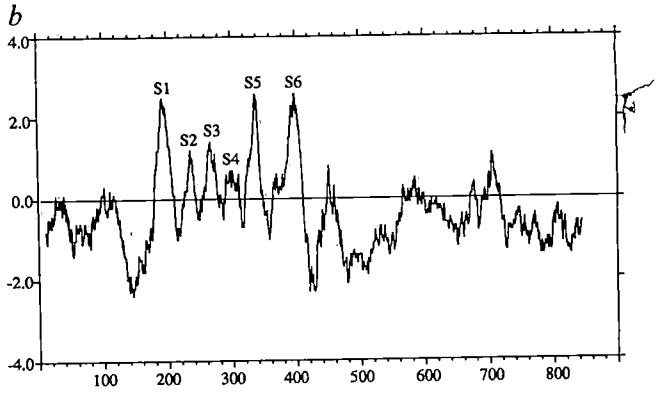

FIG. 2 *a*, Nucleotide- and deduced amino-acid sequence of *drk1*. The numbers indicate amino-acid and nucleotide positions starting at the designated initiation codon. To obtain the longest possible reading frame, the first ATG triplet was chosen as the initiation codon. The sequence flanking the second in-frame ATG (position 49–51), however, is in better agreement with the consensus sequence for translation initiation[21]. Amino acids 77–83 (heavy line on top) are conserved in all K^+ channels cloned so far. The boxes outline potential transmembrane segements S1–S6. Consensus sequences for *N*-glycosylation[22] are shown by asterisks. Only one site (position 279) faces the extracellular side in the assumed topology of the channel. Two consensus sequences for cAMP-dependent phosphorylation (●) are found in the cytoplasmic tail[17]. *b*, Hydropathy plot of the amino-acid sequence of *drk1*. The deduced amino-acid sequence of *drk1* was subjected to a hydropathy analysis according to Kyte and Doolittle, with a window size of 22 amino acids[25]. Segments S1–S6 correspond to the boxed regions in Fig. 2. Amino-acid positions are shown on the abscissa. The relative hydrophopicity index is shown on the ordinate.

METHODS. The DNA sequence of *drk1* was determined for both strands of the insert. Several restriction fragments were subcloned into M13mp19 vectors using standard recombinant DNA technology and the sequences were determined using an ABI automated DNA sequenator[23,24].

Drosophila (those encoded by *Shaker*)[6–8], rat and mouse[12–15], and to the core regions of two different putative K^+ channels encoded by the genes *Shab* and *Shaw*[18]. Figure 3 shows an alignment of the *drk1* sequence (corresponding to residues 18–430) with sequences of the *Drosophila* genes *Shaker*, *Shab*, and *Shaw* and with one of rat brain (*rck1*). In this core region, the percentage of identical amino acids in analogous positions between channels is given in Table 1. The six putative membrane-spanning segments (S1–S6) and a stretch of seven amino acids (Asn-Glu-Tyr-Phe-Phe-Asp-Arg) preceding S1 show the highest conservation within the core region of the five channels analysed. The segment S4 is probably the voltage-sensor and is characterized by a series of positively charged amino acids at every third position. The S4s of the *Shab* and *drk1* products have five positive charges; those of the *rck1* and *Shaker* products contain two additional positive flanking charges. Only four such charges

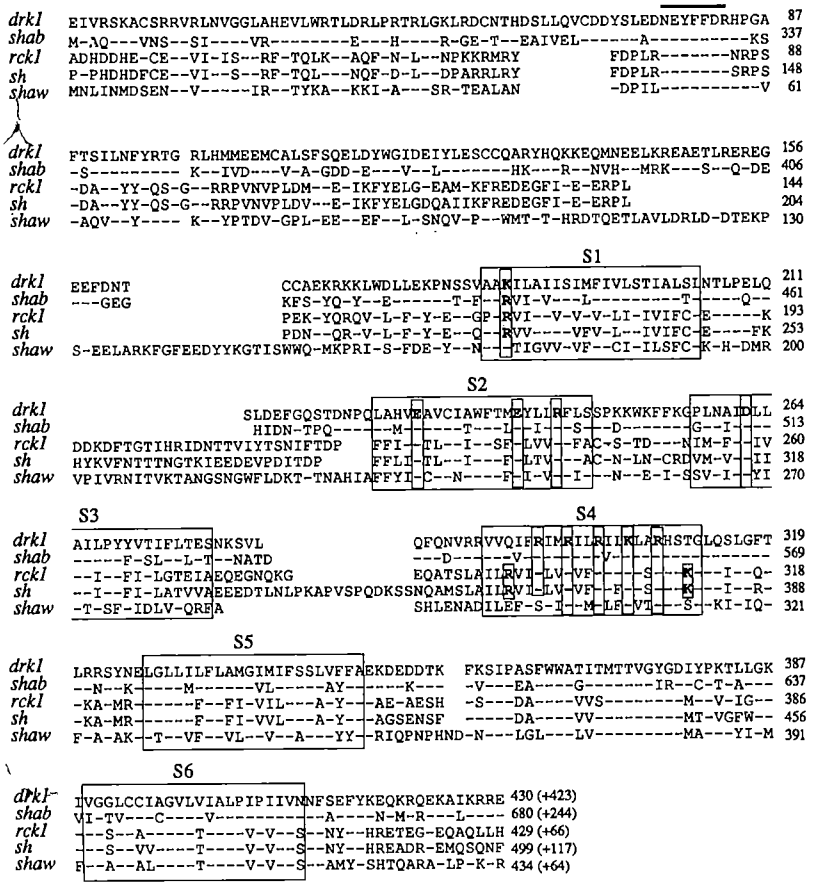

FIG. 3 Comparison of amino-acid sequences of different K^+ channels. The sequences of five different K^+ channels were aligned to reveal similarities over the areas indicated. Amino-acid positions are shown by numbers. The lengths of the missing C-termini are shown in parentheses. Identical amino acids are indicated by dashes; gaps in the sequences, by empty spaces. The *Shaker* gene encodes an A-type K^+ channel and is derived from the *Shaker*-region of *Drosophila*[4]. Two *Drosophila* cDNA clones, *Shab* and *Shaw*, whose functions are not known, are distinct from the various *Shaker* clones[18]. The gene *rck1* was isolated from a rat-brain cDNA library using a Shaker sequence as a screening probe[13]. Two families, each containing two members, are apparent. The gene *drk1* is most similar to *Shab*, whereas *rck1* is most similar to *Shaker*. The putative transmembrane regions (S1–S6) are boxed. The positive charges in the postulated voltage-sensor (S4) are shaded, as well as the conserved charged residues in other transmembrane regions. The longest stretch of identical residues present in all the channels is outlined by a heavy line. We used the EuGene software by Molecular Biology Information Resources at the Department of Cell Biology, Baylor College of Medicine, for the sequence alignment.

are present in S4 of the protein encoded by *Shaw*; this is the only (putative) K^+ channel known to have a negatively charged amino acid (glutamate) at a position where other K^+ channels have a positively charged arginine residue. It will be interesting to see whether these charge differences are responsible for some of the different kinetic properties of A-type and delayed rectifier channels. From the comparative alignment shown in Fig. 3 and Table 1 it is clear, on the basis of the conserved core sequences, that *drk1* and *Shab* are two members of the same family of genes, and *rck1* and *Shaker* are members of another family. The *drk1*/*Shab*-family and *rck1*/*Shaker* family are related and belong to a superfamily which includes a third family with *Shaw* as its only member so far. Of the *drk1*/*Shab* family only *drk1* has been expressed (this report), and we have shown that it encodes a K^+ channel with properties of a delayed rectifier. Of the *rck1*/*Shaker* family several members have been expressed. The *Shaker* clones encode A-type channels[9–11], whereas expression of *rck1* yields K^+ currents with properties of delayed rectifier-type channels[14] and possibly A-type channels[15]. The function of *Shaw* is unknown. The fact that *rck1* and *Shaker* genes are more closely related to one another than to *drk1* encoding the new K^+ channel indicates that the *rck1*/*Shaker* and *drk1*/*Shab* families are evolutionarily older than flies and mammals. The most conserved region in all K^+ channels sequenced so far is the stretch of seven amino acids near the beginning of the core. Because it is present in all types, this stretch may be a general hallmark of K^+ channels. This region seems well-suited to initiate a specific search by means of an oligonucleotide-directed screening approach to identify unknown members of the K^+ channel superfamily. □

TABLE 1 Percentage of identical amino acids in analogous positions between pairs of different K^+-channel types

	Shab	*Shaker*	*Shaw*	*rck1*
drk1 70	70	40	41	40
rck1	39	74	40	
Shaw	40	42		
Shaker	36			

Percentages were calculated using the alignment in Fig. 3. The results show that *drk1* is most closely related to *Shab*, whereas *rck1* is most similar to *Shaker*.

Received 10 May; accepted 19 July 1989.

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ACKNOWLEDGEMENTS We thank Drs Tom Caskey and Richard Gibbs, and Donna Muzny (Howard Hughes Medical Institute) for their help with automated DNA sequencing. Charybdotoxin was a gift from Drs G. J. Kaczorowski and M. L. Garcia. This work was supported in part by an Advanced Technology Program Award from the State of Texas (R.H.J.) and by the National Institutes of Health (A.M.B.).

Qa-1 restricted recognition of foreign antigen by a γδ T-cell hybridoma

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DISTINCT T-lymphocyte subsets recognize antigens in conjunction with different classes of major histocompatibility complex (MHC) glycoproteins using the T-cell receptor (TCR), a disulphide-linked heterodimer associated with the CD3 complex on the cell surface[1]. In general, class I and class II MHC products provide a context for the recognition of foreign antigens by $CD8^+$ and $CD4^+$ T cells, respectively[2]. This recognition seems to be largely dependent on αβ TCR heterodimers[3,4], whereas the function of the second γδ TCR, present on a minor subpopulation of cells, is still unknown[5]. In the mouse, the existence of six cell-surface MHC class I products (K, D, L, Qa-1, Qa-2 and Tla) has been firmly established by serological, biochemical and genetic evidence[6]. So far, only the most polymorphic of them, K, D and L ('classical' class I) have been reported as restriction elements for T-cell recognition of foreign antigens[1]. The function of the relatively invariant Qa and Tla molecules remains unknown[6,7]. We have made a T-helper cell hybridoma clone (DGT3) that recognizes synthetic copolymer poly($Glu^{50}Tyr^{50}$) in the context of Qa-1 cell surface product, and has a $CD4^-CD8^-$ phenotype. Our studies indicate that DGT3 cells express the γδ TCR on the cell surface, implicating its role in Qa-1-restricted antigen recognition. This is the first evidence that T cells can recognize foreign antigen in association with self Qa product, confirming that Qa molecules not only topologically, but also functionally, belong to the MHC.

The T-helper hybridoma clone DGT3 was obtained by fusion of poly($Glu^{50}Tyr^{50}$) (GT)-primed lymph node cells from DBA/2 ($H\text{-}2^d$) mice with the AKR ($H\text{-}2^k$) thymoma BW5147 (legend to Table 1). It reacted to GT in the absence of any additional antigen-presenting cells (APCs) (Table 1). The response was antigen-specific, because DGT3 reacted to neither of two related antigens poly($Glu^{60}Ala^{30}Tyr^{10}$) (GAT) and poly($Glu^{60}Ala^{40}$) (GA). Immunofluorescence analysis of the DGT3 clone revealed its origin (Fig. 1*a*). Strains AKR and DBA/2 carry different allomorphs of the common T-cell marker (Thy-1.1 and Thy-1.2, respectively). As DGT3 cells express Thy-1.2 determinant, they are derived from DBA/2 T cells. Like all mouse T cells, they express only class I MHC, but do not express either CD4 or CD8, suggesting that either the ancestral clone belonged to a minor subpopulation of peripheral T cells, or the expression of accessory molecules was switched off on fusion with BW5147.

As shown in Table 1, the GT specific activation of DGT3 was not inhibited by monoclonal antibodies specific for either MHC class II, 'classical' class I (K, D, L) or accessory molecules (CD4, CD8, LFA-1), although the response of the $CD4^+CD8^-$ hybridoma clone DGT1 was efficiently blocked by the antibodies specific for A^d, CD4 and LFA-1 (ref. 8). The only reagents that abolished the response of DGT3 were antibodies against $Qa\text{-}1^b$ molecules. The abrogation of DGT3 response was Qa-1 allomorph specific, because antibodies against $Qa\text{-}1^a$ (not expressed by DBA/2 or AKR strains) did not inhibit the response. As a control, anti-$Qa\text{-}1^b$ antibodies did not abolish GT specific response of the A^d restricted clone DGT1. They also did not block activation of either DGT3 with concanavalin A (con A) or the CTLL cell line with interleukin-2 (IL-2). This suggests that the mechanism of inhibition by these antibodies is not due to negative signal transmission through $Qa\text{-}1^b$ molecules, but because of interference with the presentation of GT in the context of $Qa\text{-}1^b$ by DGT3 cells themselves. This is confirmed by the experimental data displayed in Table 2. Instead of adding free soluble GT into the culture, different APCs were preincubated (pulsed) with the antigen and used to present membrane-bound GT to DGT clones. Detachment of GT from the surface of pulsed cells was prevented by glutaraldehyde fixation. DGT3 cells could be stimulated by GT pulsed APCs carrying $Qa\text{-}1^b$ but not by $Qa\text{-}1^a$ molecule, whereas their expression of different Tla or classical H-2 allomorphs was irrelevant. Neither of the class II-negative antigen-pulsed T-cell lines could activate clone DGT1, which required the presence of GT/A^d complex (displayed by B-lymphoma line A20).

It has been reported that Qa and Tla molecules are recognized by unrestricted cytotoxic T lymphocytes in the same way as other MHC antigens. Unlike classical MHC products, they are weak transplantation antigens and they have not been shown

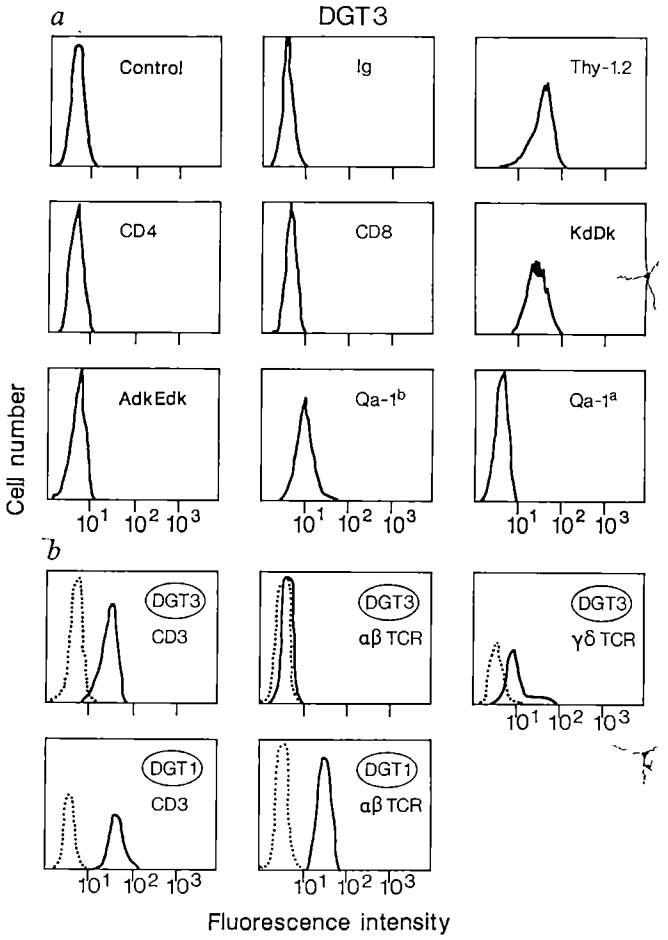

FIG. 1 Cell-surface phenotype of clone DGT3. *a*, T-hybridoma cells DGT3 were stained either directly with fluorescein (FITC) labelled antibodies against mouse immunoglobulin (Ig, Southern), Thy-1.2, CD8 (both from Becton-Dickinson) and CD4 (hybridoma GK1.5; ref. 30) or indirectly by incubation with either anti-K^dD^k (hybridoma 15.5.5S), anti-$A^{dk}E^{dk}$ (hybridoma 82.B) or antibodies against Qa-1 allomorphs (see legend to Table 1), followed by the labelling with FITC-conjugated antibodies specific for mouse Immunoglobulin (Southern). 'Control' indicates the cells incubated without addition of antibodies. *b*, DGT3 and DGT1 cells were stained indirectly by incubation with either biotin-coupled anti-CD3 monoclonal antibody (2C11; ref. 31), pan-αβTCR reactive monoclonal antibody H57.597 (ref. 12) or pan-γδTCR specific monoclonal antibody 3A10 (ref. 13) as indicated, followed by labelling with FITC-conjugated either streptavidin (for CD3; Southern) or goat antibodies specific for hamster or mouse IgG (for αβTCR or γδTCR, respectively; Southern). Broken lines represent cells stained with second step reagents only. Analysis for *a* and *b* was performed on a FACS (Becton-Dickinson) flow cytometer.

TABLE 1 Antibody blocking of response to GT antigen

Experiment	Responding cells	Antigen	A20	ConA	IL-2	Antibody (specificity)	Response (c.p.m.)
1	DGT3	—	—	—	—	—	6.265
		GT	—	—	—	—	195.089
		GT	—	—	—	15.1.5P (K^kD^k)	175.924
		GT	—	—	—	15.5.5S (K^dD^k)	199.362
		GT	—	—	—	21.460 (D^d)	198.036
		GT	—	—	—	28-14-8S (L^d)	185.596
		GT	—	—	—	polyclonal (Qa-1^a)	171.830
		GT	—	—	—	polyclonal (Qa-1^b)	42.264
		GT	—	—	—	267-503 (Qa-2^a)	172.033
		—	—	+	—	—	191.255
		—	—	+	—	polyclonal (Qa-1^b)	185.247
		—	—	—	—	—	3.528*
		—	—	—	+	—	301.097*
		—	—	—	+	polyclonal (Qa-1^a)	268.788*
		—	—	—	+	polyclonal (Qa-1^b)	281.344*
2	DGT3	—	—	—	—	—	1.002
		GT	—	—	—	—	32.699
		GT	—	—	—	129.19.6.8 (CD4)	41.490
		GT	—	—	—	H35-17.2 (CD8)	29.160
		GT	—	—	—	H68-96.22 (LFA-1)	32.902
		GT	—	—	—	8.A ($A^{d,k}$)	38.233
		GT	—	—	—	39.I ($A^{d,k}$, $E^{d,k}$)	28.671
		GT	—	—	—	82.B ($A^{d,k}$, $E^{d,k}$)	36.021
		GT	—	—	—	40.I ($A^{d,k}$, $E^{d,k}$)	37.110
		GT	—	—	—	40.B ($A^{d,k}$, $E^{d,k}$)	37.001
		GT	—	—	—	10.A ($E^{d,k}$)	29.920
		GT	—	—	—	40.D ($E^{d,k}$)	36.040
		GT	—	—	—	7.A ($E^{d,k}$)	34.667
		GT	—	—	—	74.C ($E^{d,k}$)	34.967
3	DGT3	—	—	—	—	—	591
		GAT	—	—	—	—	316
		GA	—	—	—	—	636
		GT	—	—	—	—	22.817
		GT	—	—	—	MK-D6 (A^d)	22.120
		GT	—	—	—	pool 7 (Qa-1^b)	511
		—	—	+	—	—	29.727
		—	—	+	—	pool 7 (Qa-1^b)	29.141
4	DGT1	—	—	—	—	—	1.188
		GT	—	—	—	—	1.118
		—	+	—	—	—	1.218
		GT	+	—	—	—	116.608
		GT	+	—	—	MK-D6 (A^d)	9.041
		GT	+	—	—	polyclonal (Qa-1^a)	102.444
		GT	+	—	—	polyclonal (Qa-1^b)	110.576
		GT	+	—	—	267-503 (Qa-2^a)	112.676
5	BW5147	—	—	—	—	—	1.183
		GT	—	—	—	—	1.214

Hybridoma cells (1×10^5) were cultured with or without APCs (B-cell lymphoma A20, 3×10^4 per well) and antigens. After 24 h the supernatants were collected and tested at 75% concentration for their ability to support the growth of 10^4 CTLL cells (an IL-2 dependent cell line) for 24 h. The mean values for triplicate cultures are shown.

DBA/2 mice were immunized with 100 μg GT (ref. 8). Seven days later, cells from the draining lymph nodes were cultured with 150 μg ml^{-1} GT. Surviving cells were fused with BW5147, a T thymoma whose stimulation can be assayed by the production of IL-2 (refs 21, 22). GT-specific hybridomas were selected with 150 μg ml^{-1} GT, the most effective dose for the population. The resulting hybridomas DGT3 and DGT1 (for *D*BA/2 *GT*-specific) were recloned 3 times. DGT1 was Thy-$1.2^+CD4^+CD8^-$ (ref. 8). The dose of GT, GA and GAT (Sigma) used for the assay was 150 μg ml^{-1}. All antibodies were added at the beginning of the culture period. Those specific for Qa-1 allomorphs ('polyclonal') were obtained by protein—A purification from immune antisera[23] and used at final concentration of 5 μg ml^{-1}. An independent batch of anti-Qa-1^b antiserum (designated as 'pool 7') was used at final dilution 1:30. The specificity of anti-Qa-1 antisera was determined by analysing the panel of lymphoid cells from various inbred and congenic mouse strains on a FACScan flow cytometer (Becton-Dickinson) and by inhibition of lympholysis mediated by Qa-1-specfic cytotoxic T cells (data not shown). The genetic difference between recipient and donor mice used to generate Qa-1 specific antisera also involves the *Tla* locus. Staining of peripheral mature T cells (that are Tla negative), and thymocytes expressing either the third party Tla allomorph or no Tla product at all, however, excluded possible Tla reactivity of those antisera. All monoclonal antibodies directed against class II MHC, CD4, CD8, LFA-1 and D^d (21.460) class I molecules were used at a final concentration of 5 μg ml^{-1}. Culture supernatants of hybridomas 15.1.5P and 28.14.8S (ATCC) were diluted twice. Ascitic fluids of the hybridoma antibodies 15.5.5S and 267-503 (Milan Analytica) were used at concentrations of 1%. All monoclonal antibodies were described in refs 12, 24–28. Asterisks indicate direct stimulation of CTL L line with human recombinant IL-2 (Roche).

to restrict the T-cell recognition of other antigens[6,7]. MHC-restricted recognition of foreign antigens by most of the single positive T cells ($CD4^+CD8^-$, $CD4^-CD8^+$) has been definitively attributed to the $\alpha\beta$ TCR (refs 3, 4). The majority of double negative ($CD4^-CD8^-$) peripheral T cells, however, express the $\gamma\delta$ TCR (ref. 5) and it was of interest therefore to characterize TCR gene usage in the Qa-1^b restricted double-negative DGT3 hybridoma.

Northern blot analysis of DGT3 poly(A)$^+$ RNA showed existence of all four TCR gene transcripts (α, β, γ, δ) using constant-region specific complimentary DNA probes (Fig. 2*a*). Mature-sized $\alpha\beta$ TCR messenger RNA (1.5 and 1.3 kilobases

TABLE 2 Stimulation of hybridoma clones by antigen-pulsed APCs.

Hybridoma	Soluble GT	Additional APC: non-pulsed	Additional APC: GT-pulsed	Additional APC: GT-pulsed fixed	Alleles at loci: *Tla*	Alleles at loci: *Qa-1*	Antibodies against	Response (c.p.m.)
DGT3	–	–	–	–	–	–	–	5.295
	+	–	–	–	–	–	–	83.799**
	+	–	–	–	–	–	Qa-1^a	77.911
	+	–	–	–	–	–	Qa-1^b	8.349
	–	–	BW5147	–	*b*	*b*	–	52.950
	–	–	BW5147	–	*b*	*b*	Qa-1^a	43.636
	–	–	BW5147	–	*b*	*b*	Qa-1^b	7.190
	–	–	EL4	–	(*b*)***	*b*	–	58.388
	–	–	EL4	–	(*b*)	*b*	Qa-1^a	45.864
	–	–	EL4	–	(*b*)	*b*	Qa-1^b	7.172
	–	–	R1.1	–	*a*	*a*	–	5.773
	–	–	R1.1	–	*a*	*a*	Qa-1^a	5.245
	–	–	R1.1	–	*a*	*a*	Qa-1^b	6.565
	–	–	–	BW5147	*b*	*b*	–	43.092
	–	–	–	EL4	(*b*)	*b*	–	38.166
	–	–	–	R1.1	*a*	*a*		6.388
DGT1	–	–	–	–	–	–	–	3.107
	+	–	–	–	–	–	–	2.838
	–	A20	–	–	*c*	*b*	–	4.366
	+	A20	–	–	*c*	*b*	–	103.773
	–	–	A20	–	*c*	*b*	–	95.364
	–	–	BW5147	–	*b*	*b*	–	2.996
	–	–	EL4	–	(*b*)	*b*	–	3.066
	–	–	R1.1	–	*a*	*a*	–	2.031
	–	–	–	A20	*c*	*b*	–	27.572
	–	–	–	BW5147	*b*	*b*	–	2.304
	–	–	–	EL4	(*b*)	*b*	–	3.096
	–	–	–	R1.1	*a*	*a*	–	3.235
DGT3	–	–	–	–	–	–	–	797
	+	–	–	–	–	–	–	72.722
	–	B6*	–	–	(*b*)	*b*	–	942
	–	B6-Tla^a*	–	–	(*a*)	*a*	–	1.005
	–	BALB/c*	–	–	(*c*)	*b*	–	1.214
	–	–	B6*	–	(*b*)	*b*	–	42.898
	–	–	B6-Tla^a*	–	(*a*)	*a*	–	1.117
	–	–	BALB/c*	–	(*c*)	*b*	–	32.290

IL-2 production of GT reactive hybridomas was measured as described in Table 1. EL4 and R1.1(T1a$^+$) are mouse T-cell lymphoma lines derived from C57BL6 (*H-2*^{b}) and C58 (*H-2*^{k}) strains, respectively. APCs were pulsed by 5 h coculture with GT and subsequent washing. A sample of each GT-pulsed APC line was fixed with glutaraldehyde.* Irradiated GT-pulsed splenocytes from strains B6 (*Tla*^{b}, *Qa*-1^b), B6-Tla^a (*Tla*^{a}, *Qa*-1^a) and BALB/c (*Tla*^{c}, *Qa*-1^b) were used at final concentration of 5×10^5 per well. ** Significant responses are underlined.*** Tla antigen is not expressed[6,29].

(kb), respectively) probably originated from the fusion partner BW5147 thymoma, as deduced from the Southern blot analysis. Clone DGT3 had the same two V-D-Jβ2 rearrangements as its lymphoma ancestor BW5147 and an additional two in the germline configuration as judged by the position and intensity of the 5′-Dβ2/Jβ2-specific bands on the autoradiograph (data not shown). The Cβ1 genes were either in germline or not completely rearranged, because both the Cβ and 5′-Dβ1 probes detected two superimposable bands on Southern blots containing *Hin*dIII-digested DNA (data not shown). Thus, only the BW5147-specific V-D-Jβ-rearrangements could be found in the DGT3 clone. In the Southern blot, the Cδ probe hybridized to DGT3 but not to BW5147 genomic DNA, detecting in addition to the 30 kb germ-line fragment, a smaller rearranged *Bam*HI DNA fragment (Fig. 2*b*). Because *Bam*HI sites flank the Cδ gene, the double band suggests the presence of a Cδ-containing pair of chromosomes in the DGT3 genome. This implies that only BW5147-specific α-gene rearrangements should be present in DGT3 cells (if they do not have more than four copies of chromosome 14), as α and δ TCR gene rearrangements seem to be mutually exclusive. The δ TCR chain transcripts in DGT3 appear as bands migrating at ~1.8 kb and ~1.5 kb, neither of which are detected in DGT1 cells (Fig. 2*a*). BW5147 carries three out-of-frame γ-gene rearrangements, only one of which seems to be transcribed (γ1 gene; refs 9–11). In Southern blots with *Eco*RI-digested DNA, compound with the characteristic pattern of BW5147, DGT3 showed an additional rearranged band of 5 kb with the Cγ1-probe (Fig. 2*b*). This suggests the existence of an additional γ-gene product in DGT3 cells, not normally found in BW5147 cells.

The DGT3 specificity (GT/Qa-1^b) could not be attributed to $\alpha\beta$ TCR, because the GT/A^d specific hybridoma DGT1 was not activated by GT in association with Qa-1^b, excluding the involvement of BW5147 $\alpha\beta$ TCR (Table 1, experiment 4). Also, DGT3 cells neither stained with pan-$\alpha\beta$ TCR reactive monoclonal antibody (H57.597; Fig. 1*b*; ref. 12) nor contained any other functional β-chain rearrangement other than the BW5147 type (not shown), excluding, in addition, any contribution by $\alpha\beta$ chains to the DGT3 TCR. The DGT3 clone expressed CD3 complex on the cell surface, presumably in association with $\gamma\delta$ TCR (Fig. 1*b*). The presence of the TCR heterodimer was demonstrated by staining DGT3 cells with pan-$\gamma\delta$ TCR reactive monoclonal antibody (3A10, Fig. 1; ref. 13) and SDS-PAGE of membrane-labelled DGT3 cell lysates, immunoprecipitated with antiserum specific for Cγ1/4. As shown in Fig. 2*c*, anti-Cγ1/4 antiserum, but not pan-$\alpha\beta$ TCR specific monoclonal antibody or anti-Cγ2 antiserum, immunoprecipitated from DGT3 cells a heterodimeric molecule with relative molecular masses ~34–36 and ~42–44 K corresponding to $\gamma\delta$ TCR (ref. 5). From the DGT1 clone, pan-$\alpha\beta$ TCR reactive monoclonal antibody precipitated a different heterodimeric molecule as compared with DGT3. Taken together, our results indicate that DGT3 cells

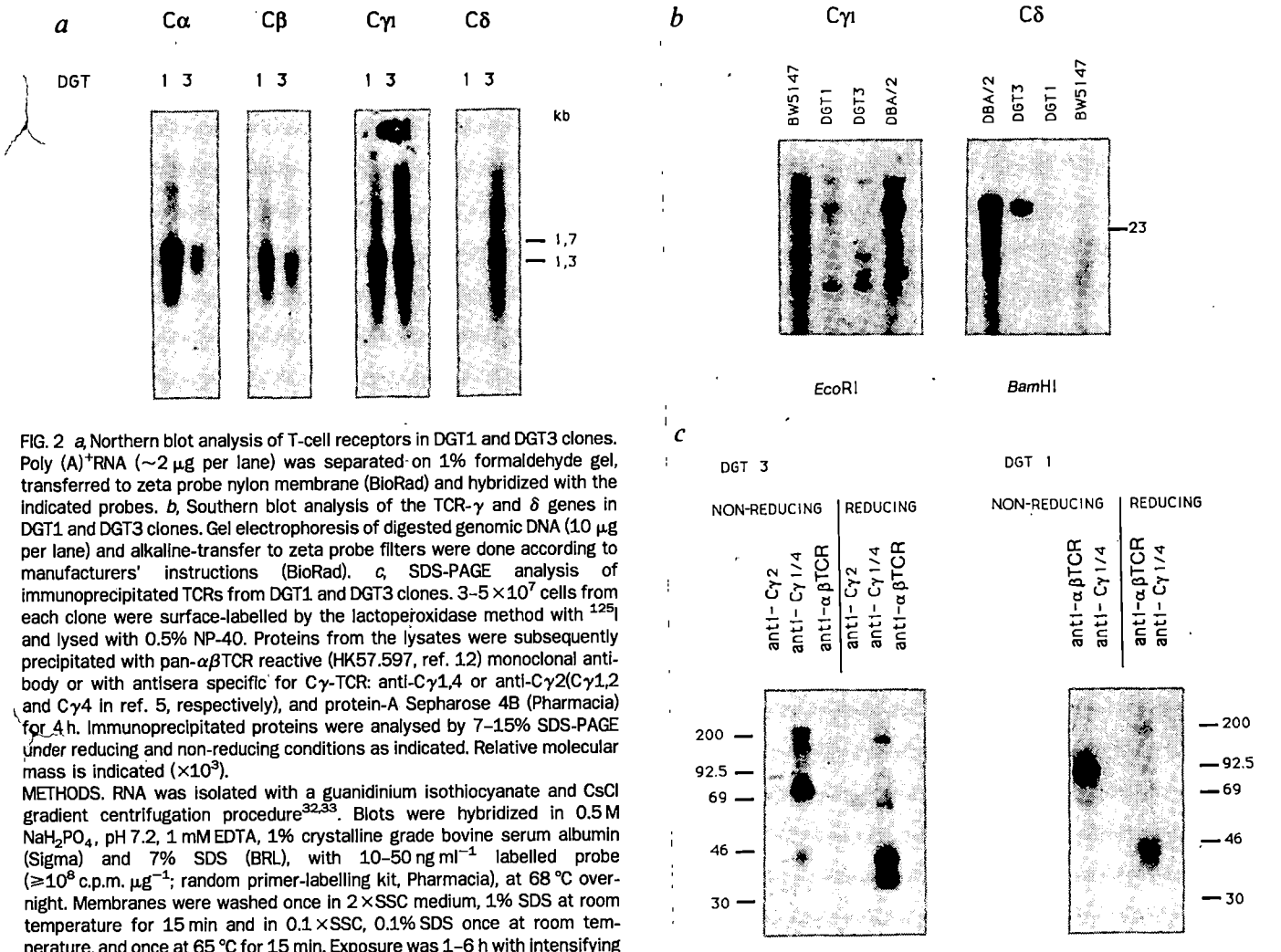

FIG. 2 *a*, Northern blot analysis of T-cell receptors in DGT1 and DGT3 clones. Poly $(A)^+$RNA (~2 μg per lane) was separated on 1% formaldehyde gel, transferred to zeta probe nylon membrane (BioRad) and hybridized with the indicated probes. *b*, Southern blot analysis of the TCR-γ and δ genes in DGT1 and DGT3 clones. Gel electrophoresis of digested genomic DNA (10 μg per lane) and alkaline-transfer to zeta probe filters were done according to manufacturers' instructions (BioRad). *c*, SDS-PAGE analysis of immunoprecipitated TCRs from DGT1 and DGT3 clones. $3–5 \times 10^7$ cells from each clone were surface-labelled by the lactoperoxidase method with ^{125}I and lysed with 0.5% NP-40. Proteins from the lysates were subsequently precipitated with pan-αβTCR reactive (HK57.597, ref. 12) monoclonal antibody or with antisera specific for Cγ-TCR: anti-Cγ1,4 or anti-Cγ2(Cγ1,2 and Cγ4 in ref. 5, respectively), and protein-A Sepharose 4B (Pharmacia) for 4 h. Immunoprecipitated proteins were analysed by 7–15% SDS-PAGE under reducing and non-reducing conditions as indicated. Relative molecular mass is indicated ($\times 10^3$).

METHODS. RNA was isolated with a guanidinium isothiocyanate and CsCl gradient centrifugation procedure[32,33]. Blots were hybridized in 0.5 M NaH_2PO_4, pH 7.2, 1 mM EDTA, 1% crystalline grade bovine serum albumin (Sigma) and 7% SDS (BRL), with 10–50 ng ml^{-1} labelled probe ($\geq 10^8$ c.p.m. μg^{-1}; random primer-labelling kit, Pharmacia), at 68 °C overnight. Membranes were washed once in 2 × SSC medium, 1% SDS at room temperature for 15 min and in 0.1 × SSC, 0.1% SDS once at room temperature, and once at 65 °C for 15 min. Exposure was 1–6 h with intensifying screens (Ilford Fast Tungstate). Sizes were estimated from the ethidium bromide-stained DriGest III marker (Pharmacia) run in parallel on the same gel. All probes were gel purified. Probe Cα is the 200 bp *Eco*RV-*Ava*II fragment of the cDNA clone T1.2 (ref. 34); Cβ probe is the 300 bp insert of the β-chain clone 4.4 described previously[35]; Cγ1 is the 1.25 kb *Kpn*I–*Eco*RI fragment of the insert of the clone pTBDγ-4 isolated from the BDFL T-cell clone cDNA library (contains half of the Vγ1 entire Cγ and 3′ untranslated region)[9]; Cδ is the 900 bp *Eco*RI insert of the δ-chain specific cDNA clone described previously[36]. Immunoprecipitations and SDS-PAGE analysis were according to ref. 37.

express γδ TCR heterodimers on the cell surface, and these may play a role in the Qa-1 restricted recognition of GT.

This is the first report of Qa-associated recognition of foreign antigen by T cells. The question remains whether the recognition event has a physiological significance *in vivo*. The DGT3 specificity might originate from a minor population of T lymphocytes whose antigen recognition was Qa-1-dependent. Double-negative γδ TCR mature cytotoxic T-cell clones have broad, MHC-unrestricted specificity[14–16]. Some of them seem to recognize allogeneic Tla and D class I molecules[14]. Although DGT3 had no crossreactivity to eight different common H-2 haplotypes (data not shown), it is still possible that this was not detected, because of an insufficiently large panel of potential stimulators. Conversely, the reported broad specificity of 'double negative' clones might represent crossreactivity of Qa-restricted T cells specific for unknown foreign antigen(s). It has recently been shown that the recognition of foreign antigens by human γδ T cells seemed to be restricted by monomorphic HLA determinants[17–19], but it remained unclear whether MHC restriction elements were encoded by the 'classical' or other ('non-classical') HLA genes[20]. It might be possible, therefore, that in these cases foreign antigens were recognized in association with elusive human counterparts of mouse Qa-1 antigens. It is conceivable that the possible physiological role of Qa-1-restricted recognition is not too distant from class I- or class II-restricted recognition by T cells. In contrast to previous findings, Qa-1 antigens were found to be widely expressed, in a similar way to classical class I molecules[7]. Thus, they might provide a context for the presentation of antigens for the 'first line of defence' T lymphocytes. Whether these cells bear only γδ TCRs remains to be answered. □

Received 27 March; accepted 17 July 1989.

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ACKNOWLEDGEMENTS. We thank Marie-Françoise Beya and Wendy Marstone for help with SDS-PAGE, Drs Lorraine Flaherty and Drew Pardoll for antisera, Drs Ralph Kubo, Michel Pierrés and S. Tonegawa for monoclonal antibodies, Dr Klaus Karjalainen for δ TCR gene probes, and Drs Harald von Boehmer, James Kaufman, Polly Matzinger and Michael Steinmetz for discussion and manuscript review. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-La Roche & Co. Ltd.

Embryonic MAP2 lacks the cross-linking sidearm sequences and dendritic targeting signal of adult MAP2

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THE most prominent microtubule-associated protein of the neuronal cytoskeleton is MAP2. In the brain it exists as a pair of high-molecular weight proteins, MAP2a and MAP2b, and a smaller form, MAP2c, which is particularly abundant in the developing brain[1–3]. High-molecular weight MAP2 is expressed in dendrites, where its messenger RNA is also located[4,5], but is not found in axons[6–9]; it has been shown to be present in fine filaments that crosslink dendritic microtubules[10]. This correlates with the primary structure of high-molecular weight MAP2, which consists of a short carboxy-terminal tubulin-binding domain and a long amino-terminal arm[11], which forms a filamentous sidearm on reconstituted microtubules[12–14]. Here we report that the high- and low-molecular weight forms of MAP2 are generated by alternative splicing and share the entire C-terminal tubulin-binding domain as well as a short N-terminal sequence. In contrast to high molecular weight MAP2, embryonic brain MAP2c lacks 1,342 amino acids from the filamentous sidearm domain. Furthermore, the mRNA for low molecular weight MAP2c is not present in dendrites, indicating that the dendritic targeting signal is specific for the high-molecular weight form.

We have previously described a MAP2 complementary DNA clone, 38a, which hybridizes to the 6-kilobase (kb) mRNA that encodes MAP2c (ref. 3). The nucleotide sequence of this clone was determined and the deduced amino-acid sequence was compared with that of high-molecular weight MAP2 from juvenile brain[11]. The alignment of the deduced primary structures showed that MAP2c consists of a sequence from the N-terminus of high molecular weight MAP2 joined to a sequence from the MAP2 C-terminus (Fig. 1). There is >95% amino-acid sequence similarity between rat MAP2c and the appropriate 5′ and 3′ portions of mouse MAP2. Allowing for inter-species variation, this suggests identity between the MAP2c sequence and their counterparts in high-molecular weight MAP2. The C-terminus of MAP2c thus contains all 3 of the 18-amino-acid repeated sequences that constitute the tubulin-binding domain of high-molecular weight MAP2[11].

To confirm that the sequence arrangment seen in clone 38a genuinely reflects the structure of MAP2c, we prepared cDNA probes from the 3′, 5′ and the joining regions of clone 38a, and used them to demonstrate their relationship to the 6-kb MAP2c and the 9-kb MAP2 mRNAs by northern blot analysis (Fig. 2). Figure 2*a* shows that both the 5′ and 3′ ends of clone 38a independently hybridized to the 6-kb MAP2c mRNA and also to the 9-kb MAP2 mRNA, confirming that MAP2c contains both N- and C-terminal sequences of the high-molecular weight MAP2. A northern blot of the same mRNA species with rat cDNA clone 19a (ref. 3), which encodes part of the sidearm domain of high-molecular weight MAP2, shows hybridization only to the 9-kb mRNA (Fig. 2*a*), confirming that this sequence is absent from MAP2c. In contrast, a synthetic oligonucleotide probe (31-mer) spanning the joining region 'J' of clone 38a hybridized specifically to the 6-kb MAP2c mRNA (Fig. 2*b*). As a control we subsequently hybridized the same northern blot with clone 19*a* without removing the 'J' probe, to reveal the 9-kb MAP2 mRNA (Fig. 2*b*). A 64-mer oligonucleotide probe derived from 5′-untranslated sequence of a rat high-molecular weight MAP2 clone also hybridized to both the 6-kb- and 9-kb mRNAs (data not shown), indicating that the sequence identity between high- and low-molecular weight MAP2 transcripts

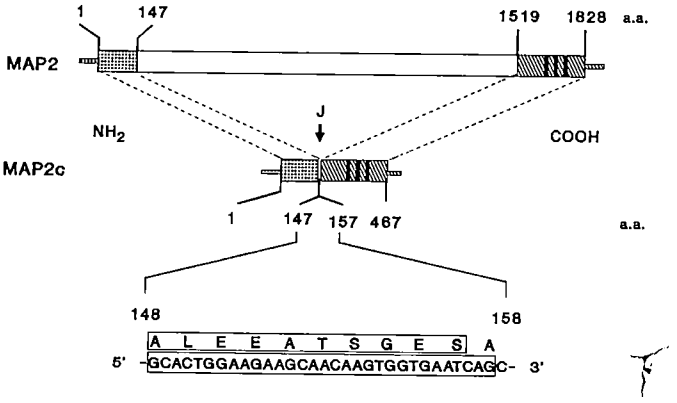

FIG. 1 Schematic representation of mouse high-molecular weight MAP2 and rat MAP2c protein sequences drawn to scale. Shaded boxes represent the N- and C-terminal amino-acid regions that are common to both proteins and are respectively 95% and 99% similar. The high sequence homology continues within the 3′ and 5′-non-coding DNA-sequences adjacent to the open reading frames, showing 87% similarity at the 5′ and 95% at the 3′ end over 67 and 173 nucleotides, respectively. The three vertical black boxes indicate the repetitive sequences of the tubulin-binding domains[10]. The dotted lines indicate the alternative splicing of the primary transcript to give either MAP2 or MAP2c, with the result that 1,372 amino acids of the filamentous sidearm-domain (open box) are absent from MAP2c. The nucleotide sequence spanning the joining region J of MAP2c and the deduced amino-acid sequence are also shown. The sequence of the oligonucleotide used for northern blot hybridization (nucleotides 307–342) and the amino-acid sequence of the synthetic decapeptide used to raise antibodies are boxed. Single letter code: A, Ala; L, Leu; E, Glu; T, Thr; S, Ser; G, Gly.
METHODS. The cDNA of the λ38a (ref. 10) clone was subcloned into the *Eco*RI site of the Bluescipt KS vector (Stratagene). The complete sequence was determined for both strands by dideoxy sequencing (Sequenase kit, United States Biochemical) using synthetic internal primers. DNA sequences were analysed using the software package from the University of Wisconsin Genetics Computer Group[24] and the FastP and FastA programs run on a Vax 8600.

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extends to the region upstream of the translation initiation codon.

As final confirmation that 38a is a MAP2c cDNA, and to demonstrate that the splicing region is present in the MAP2c protein, we raised an antiserum against a synthetic decapeptide (see Fig. 1) containing the joining-region sequence. Figure 2*c* shows western blots of juvenile rat brain supernatant, stained with either a monoclonal antibody against MAP2 that recognizes all the MAP2 forms (lane C) or the antibody against the decapeptide of the MAP2c joining region (lane J). Whereas the anti-MAP2 antibody strongly stained the high-molecular weight MAP2 form, the antibody against the MAP2c joining sequence selectively stained MAP2c.

Previous *in situ* hybridization experiments with a probe specific for the 9-kb MAP2 mRNA showed that this transcript is present in dendrites[4,5]. We used the 31-mer oligonucleotide probe corresponding to the MAP2c joining region (J probe) to localize the 6-kb MAP2c mRNA *in situ*. Figure 3 shows *in situ* hybridization patterns with the J probe in the adult rat entorhinal cortex. Unlike the control C-sense oligonucleotide, the J probe hybridized in a punctate pattern characteristic of the distribution of cortical pyramidal neurons. There was no significant hybridization with the J probe in pyramidal cell primary dendrites, nor in layer I, where the high-molecular weight MAP2 mRNA is found[5].

In both the developing (Fig. 4*c*) and the adult hippocampus (Fig. 4*f*), the J probe hybridized with neuronal cell bodies and not the dendrite-rich molecular layers of the hippocampal pyramidal cells and the granule cells of the dentate gyrus. In contrast, the 9-kb mRNA was found in these molecular layers (Fig. 4*a*, *e*). Figure 4 also demonstrates the necessity of using the oligonucleotide probe to locate the MAP2c mRNA: the patterns of hybridization with 38a and 19a cDNA clones are indistinguishable (Fig. 4*a*, *b*).

Our results demonstrate that the low-molecular weight, embryonic brain form of MAP2 consists of the extreme N-terminal and C-terminal ends of the high-molecular weight MAP2 coding sequence. A large portion of the central region of high-molecular weight MAP2, constituting most of the sidearm domain that projects from and may crosslink microtubles, is missing. The primary structure of MAP2c, together with the evidence that both the 9-kb and 6-kb MAP2 mRNAs are derived from a single MAP2 gene[3,17], indicate that high- and low-molecular weight MAP2s are the result of alternative splicing of a primary MAP2 gene transcript. The embryonic MAP2c results from the splicing out of a sequence coding for 1,372 amino acids of the central domain of mature MAP2. This alternative splicing is developmentally regulated, for MAP2c and its 6-kb mRNA are more abundant during brain development than in the adult brain[1,3]. In the rat[1], quail[18] and *Xenopus*[19], the disappearance of MAP2c occurs abruptly, and coincides with the stabilization of neuronal morphology.

The expression of the various MAP2 forms is temporally as well as spatially regulated. High-molecular weight MAP2 is present in dendrites[6-9], where its 9-kb mRNA is also localized[4,5], in contrast to MAP2c, which is found in axons[18,19]. This suggests that high-molecular weight MAP2 contains unique sequences, absent from MAP2c, that are responsible for its specific expression in dendrites and not in axons. The presence of the 9-kb high-molecular weight MAP2 mRNA in dendrites further

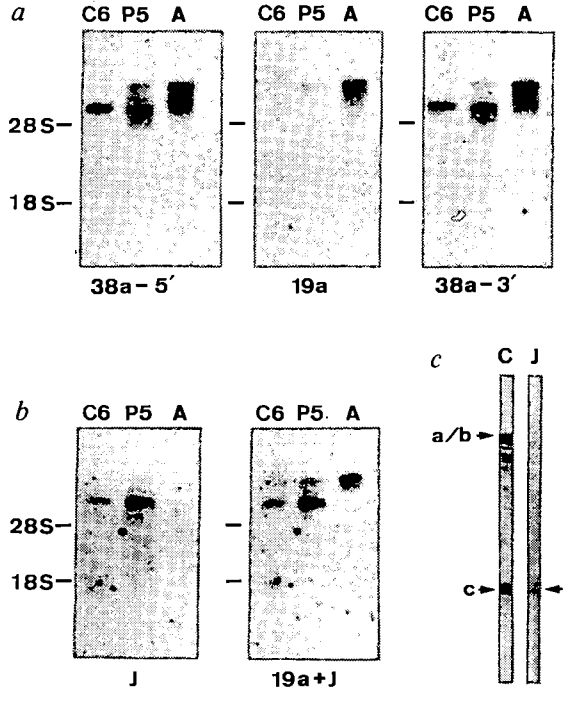

FIG. 2 Verification of the MAP2c and high-molecular weight MAP2 transcript structure by northern blot analysis. Northern blot hybridization of poly(A)$^+$ mRNA from C6 glioma cells (C6), postnatal day-5 (P5) baby rats and adult rats (A). *a*, DNA-sequences from the 3′-end (left) and the 5′-end (right) of MAP2c, and from the (high molecular weight) MAP2-specific region encoding the filamentous sidearm (clone 19a (ref. 3), middle) were used to probe the blots. *b*, Northern blot hybridization (left) with the synthetic oligonucleotide from the joining region J of MAP2c (Fig. 1*c*). The same blot was subsequently hybridized in addition with the high-molecular weight MAP2-specific clone 19a to confirm the presence of the 9-kb mRNA that does not hybridize with the J probe (right). The positions of 18S and 28S ribosomal RNAs are indicated. *c*, Immunostaining of heat-stable MAPs from P6 (postnatal day 6) rat brain-supernatant with the antiserum against the MAP2c J peptide. The blot on the left was stained with the C monoclonal antibody that recognizes both high- and low-molecular weight MAP2 (arrows). The blot on the right shows the staining with the mouse polyclonal serum raised against the joining peptide J (see Fig. 1*c*), which reacts selectively with MAP2c (arrow).

METHODS. For northern blot analysis, poly(A)$^+$ RNA was denatured by glyoxal at 50 °C, electrophoresed on 1% agarose gels and transferred onto Hybond-N (Amersham) nylon membranes. The RNA was fixed by UV irradiation. Prehybridization was at 42 °C in 50% formamide, 5×SSC, 50 mM sodium phosphate, *p*H 6.8, 0.5% SDS, 10% dextran sulphate and 200 μg ml^{-1} heat denatured heterologous DNA. Hybridization was carried out in the same solution containing 2×10^6 c.p.m. ml^{-1} of heat-denatured probe. The cDNA fragments were labelled by random oligonucleotide priming. Filters were washed twice for 20 min in 2×SSC, 0.1% SDS at 60 °C, and for 20 min in 0.2×SSC, 0.1% SDS at 60 °C. The probes used for hybridization were: a 230-basepair (bp) *Eco*RI–*Pst*I fragment from the 3′-end of clone 38a, spanning amino acids 170–466; a 1,064-bp *Tth*I–*Eco*RI fragment from the 5′-end encoding amino acids 45–121; and the 1,313-bp *Eco*RI insert of clone 19a. Hybridization with [γ-^{32}P]dATP (2×10^6 c.p.m. ml^{-1})-kinased oligonucleotide (31-mer) was performed at 40 °C in 6×NET, 5×Denhardt's solution, 0.5% SDS and 200 μg ml^{-1} transfer RNA. Filters were washed twice, for 20 min with 2×SSC and 0.1%SDS at 50 °C and for 15 min with 0.2×SSC and 0.1% SDS at 37 °C. The decapeptide of the MAP2c joining region (Ala-Leu-Glu-Glu-Ala-Thr-Ser-Gly-Glu-Ser) was synthesized by the Fmoc-polyamide method[25] on a CRB-pepsynthesizer. The peptide was coupled to keyhole-limpet haemocyanin before immunization. Production of antiserum in mouse and immunoblotting were performed as described[3]. The serum was diluted 1:100 and the supernatant of the antibody against MAP2 that recognizes all the MAP2 forms diluted 1:10.

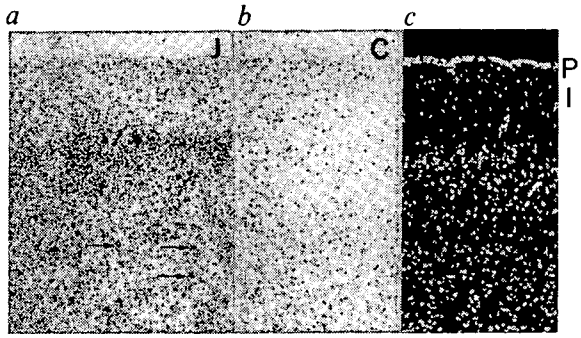

FIG. 3 *In situ* hybridization of the oligonucleotide spanning the MAP2c joining region to coronal sections through the entorhinal cortex of an adult rat brain. *a*, The bright field image shows the distinctive punctuate hybridization pattern in the cell bodies of neurons in the cortex obtained with the 31-mer J oligonucleotide. Layer I (I) and the pia (P) show no significant hybridization (see *c*). *b*, Hybridization of an adjacent section with a control 27-mer sense *C*-oligonucleotide gave only uniform background labelling. *c*, Location of cells bodies shown by staining with Hoechst nuclear dye.

METHODS. *In situ* hybridization using the MAP2 (19a) and MAP2c (38a) cDNA inserts was as described previously[4,5]. For hybridization with the 31-mer MAP2c-J oligonucleotide and the 27-mer control sense-probe the sections were thawed and prehybridized for 1 h at 37 °C (in 25–40 μl per slide) in 50% formamide, 4 × SSC, 20 mM sodium phosphate, *p*H 6.8, 1 × Denhardt's solution, 0.2% SDS, 10% dextran sulphate, 20 mM β-mercaptoethanol and 100 μg ml^{-1} each of heat-denatured single-strand DNA, tRNA and poly(A) DNA. The hybridization was carried out overnight in the same solution containing 1 × 10^6 c.p.m. per 100 μl of [α-^{35}S]dATP-labelled probes. The sections were then washed three times for 20 min in 1 × SSC at room temperature, three times for 20 min in 1 × SSC at 37 °C and twice for 20 min in 0.5 × SSC at room temperature, and dehydrated. Following exposure to β-max (Amersham) film for 1–3 days, the slides were dipped in K5 nuclear emulsion (Ilford) and exposed for 7–10 days. The sections were further processed as described previously[8].

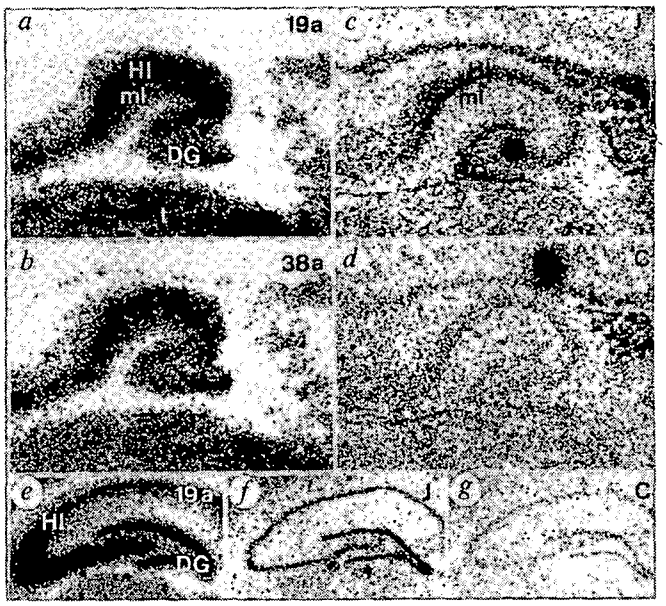

FIG. 4 Localization of the 9-kb and 6-kb mRNAs of MAP2 in the developing (*a–d*) and adult (*e–g*) rat hippocampus. The hybridization patterns in the developing hippocampus are indistinguishable with the two cDNA probes 19a (*a*) and 38a (*b*); both show diffuse hybridization in the molecular layer (ml) of the hippocampal pyramidal cells (HI) and in the dentate gyrus (DG). The 6-kb-specific J oilgonucleotide probe (*c*) hybridizes only within the cell-body layers of the hippocampus and dentate gyrus. Sections hybridized with a control oligonucleotide C (*d*) show a background staining mainly within the molecular layer. A similar segregation of high- and low-molecular weight MAP2 mRNAs is seen in the adult. The 19a cDNA (*e*) hybridizes with a diffuse pattern in both the hippocampus (HI) and dentate gyrus (DG), in contrast to the 6-kb-specific J oligonucleotide (*f*), which hybridizes specifically to the neuronal cell bodies,. The sense oilgonucleotide C (*g*) was used as a control.

suggests that the dendritic targeting signal may be present in the mRNA itself. These sequences could be related to the long central portion of high-molecular weight MAP2 that is missing from MAP2c, or alternatively could reside in 5′ or 3′ non-coding regions of the MAP2 transcript. Examples of controlled cytoplasmic distribution of mRNAs based on 3′-untranslated sequences have recently been described for maternal transcripts in *Xenopus* oocytes[20] and *Drosophila* embryos[21].

We have recently found that MAP2c is persistently expressed in areas of the adult brain where neuronal growth continues. Levels of MAP2c remain high in retinal photoreceptor cells[22] that regenerate their outer segments, and in the olfactory bulb, where neurite outgrowth and synaptogenesis persist in the adult[23]. Thus the expression of MAP2c seems to be important for neuronal morphogenesis. Because MAP2c is essentially high-molecular weight MAP2 with 1,372 amino acids missing from the middle, its selective expression during neuronal differentiation is probably related to the function of this central portion. The significance of this enormous deletion may be related to the demonstrated involvement of the high-molecular weight MAP2 'sidearm' domain in crosslinks between microtubules[10]. Because the isolated C-terminal repeats of MAP2 have been shown to bind to tubulin polymers[11], MAP2c, which contains the entire MAP2 tubulin-binding domain, should bind to microtubules with the same efficiency as high-molecular weight MAP2. In the developing neuron, MAP2c would thus occupy MAP2 binding sites on microtubules without providing the extended sidearm domain. One possible consequence of this is that the expression of MAP2c reduces the degree of cytoskeletal crosslinkage during axonal and dendritic growth, thus providing a more plastic structural state during neuronal morphogenesis. The maturation of neuronal shape would then involve the disappearance of MAP2c and the appearance of an additional high-molecular weight MAP2, MAP2a[1,15,16], to provide additional crosslinks in the neuronal cytoskeleton and hence stabilize the adult form of the dendrites. □

Received 22 June; accepted 24 July 1989.

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ACKNOWLEDGEMENTS. We thank A. Wallace for synthesizing the oligopeptide and for computing advice and L. Farmer and C. Viereck for their help in raising the antiserum and immunoblotting.

Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity

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BOTH thyroid hormone (T_3) and retinoic acid signal essential steps in development, differentiation and morphogenesis. Specific nuclear receptors for these ligands have recently been cloned[1–8]. Previously we have noted a close homology between the DNA-binding domains of the ε-retinoic acid receptor (RAR-ε, also designated RAR-β), the thyroid hormone receptors and the oestrogen receptor[2,9]. We have now found that RAR-ε is very efficient at inducing transcription from two distinct thyroid-hormone responsive elements (TREs). Transcription induced by ligand-activated RAR-ε from a TRE can, however, be repressed by thyroid-hormone receptor in the absence of its ligand. Conversely, in the presence of its ligand, thyroid-hormone receptor will activate transcription from a TRE irrespective of the presence of unbound RAR. The use of hybrid receptors has shown that the DNA-binding domain of RAR is the essential target for inhibition by thyroid-hormone receptors. These data, together with *in vitro* DNA-binding studies, suggest that thyroid-hormone receptors may have dual regulatory roles: in the presence of hormone they function as TRE-specific transcriptional activators; in the absence of hormone, however, they can function as TRE-specific repressors.

We investigated whether TREs and/or the oestrogen-responsive element (ERE) could be activated by RAR-ε. The transcriptional activition of reporter genes of these elements was monitored using constructs in transient transfection assays. The constructs contained the chloramphenicol acetyltransferase reporter gene (*CAT*) linked to the TRE of the myosin heavy chain gene (*mhc*)[10], the synthetic palindrome TRE (G17-2)[11] or the ERE of the vitellogelin gene[12]. Co-transfection of F9 teratocarcinoma cells with a RAR-ε expression vector and either of the two TRE-*CAT* constructs resulted in the induction of CAT activity that was dependent on the presence of retinoic acid. By contrast, retinoic acid did not significantly stimulate CAT activity when the RAR-ε expression vector was co-transfected with the ERE-*tk*-*CAT* construct (Fig. 1*a*). In the presence of retinoic acid, both the TRE constructs yielded very similar levels of CAT activity. In the absence of retinoic acid, however, CAT activity was constitutively stimulated at a high level by the *mhc*-TRE-CAT construct, but at a low basal level by the G17-2-CAT construct. The G17-2 construct, therefore, behaved as an ideal retinoic-acid inducible gene, in that an increase in CAT activity induced by retinoic acid of more than 30-fold was observed (Fig. 1). When the thyroid hormone α-receptor (TR-α) was co-transfected with the reporter constructs in the presence of T_3, similar levels of CAT activity were obtained (Fig. 1*a*).

F9 cells have been established as a cellular model for retinoic-acid dependent differentiation[13]. We therefore investigated whether the endogenous F9 RAR could also activate the TRE-CAT genes by transfecting F9 cells with the reporter genes alone. A 12-fold increase in CAT activity was induced in the presence of retinoic acid on transfection with the G17-2 construct. This was considerably lower than the increase observed when F9 cells were co-transfected with RAR-ε and the G17-2 construct, indicating that the endogenous RAR is limiting in concentration. The high basal level of CAT activity observed with the *mhc*-TRE construct prevented observation of a clear induction effect by

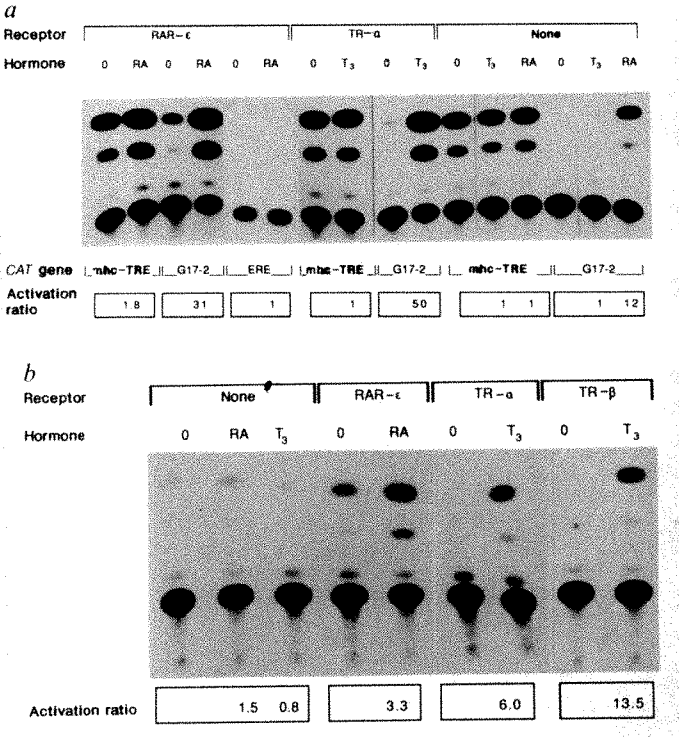

FIG. 1 RAR-ε and the endogenous F9 cell RAR activate transcription from thyroid-hormone responsive elements. *a*, F9 cells were co-transfected[2] with 5 μg expression vectors PECE-RAR-ε or PECE-TR-α and 20 μg reporter plasmids G17-2-CAT or MHC-CAT. As a control, cells were also transfected with the reporter plasmids only. The G17-2-CAT construct contains three copies of a synthetic TRE derived from the rat growth-hormone gene[19]. The *mhc* construct contains nucleotides −163 to −81 upstream of the CAP site of the rat α-*mhc* gene[10]. Activation ratio is the ratio of relative CAT activity after hormone induction to relative CAT activity without hormone induction. The mean of at least three experiments is given. *b*, RAR-ε activates transcription from a TRE in CV-1 cells. Cells were co-transfected with PECE-RAR-ε, PECE-TR-α or PECE-TR-β (5 μg each) as indicated, and G17-2-CAT construct (20 μg). Final concentrations of hormone were: retinoic acid (RA), 6×10^{-7} M; and T_3, 10^{-7} M. Mean activation for three separate experiments is given.

METHODS. F9 cells (2×10^6) were cultured as a monolayer on gelatine-coated dishes in alpha-ME medium (GIBCO), supplemented by 10% FCS, glutamine and nucleosides as described[20]. Cells were fed the medium containing charcoal-treated FCS, 2–5 h before transfection; 24 h after transfection, cells were fed with charcoal-treated medium together with the appropriate amount of hormone (final hormone concentrations, 5×10^{-8} M); 48 h after transfection, cells were collected, lysed and assayed as described[21]. CV-1 cells were plated at 1.8×10^6 per dish 12–16 h before transfection in DMEM medium (GIBCO) and 10% fetal calf serum. For accurate determinations of chloramphenicol acetylation, the separated reaction products were cut out from silica plates and quantified in a scintillation counter. Plasmid constructions: PECE-RAR-ε was obtained by cloning a *Bam*HI–*Eco*R1 fragment (containing the complete coding region of RAR-ε) from clone BI-RAR (ref. 2) (BI designates BlueScript; Stratagene) into the *Bgl*II and *Eco*R1 sites of the PECE expression vector[22]. To obtain PECE-TR-α, a 2-kilobase (kb) *Eco*R1 fragment of the TR-α clone prbeA12[5] was cloned into the *Eco*R1 site of PECE (ref. 22). For construction of PECE-TR-β, a 1.6-kb *Eco*R1 fragment of the human c-*erb*-*A*-β clone[4] was ligated into the *Eco*R1 site of the PECE vector. G17-2-CAT was derived from G17-2 Luc (C. K. Glass *et al.*, manuscript in preparation) by exchange of the reporter gene.

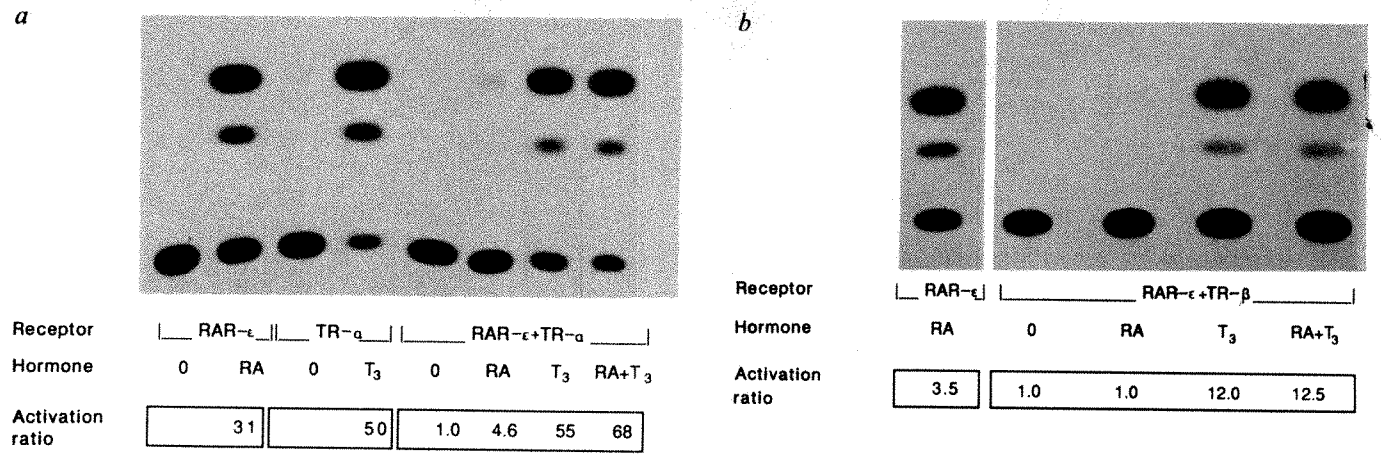

FIG. 2 TRs repress the activity of RAR-ε in F9 and CV-1 cells. *a*, PECE-RAR-ε (5 μg) and PECE-TR-α (5 μg) were co-transfected together with G17-2-CAT (20 μg) into F9 cells. As a control, each receptor was also co-transfected with G17-2-CAT individually. Hormones were added as indicated. *b*, PECE-TR-β (2.5 μg) and PECE-RAR-ε (2.5 μg) were co-transfected with G17-2-CAT (20 μg) into CV-1 cells. As a control, PECE-RAR-ε was also co-transfected with G17-2 individually. Hormones were added as indicated. CAT activity was assayed and quantified as described in Fig. 1.

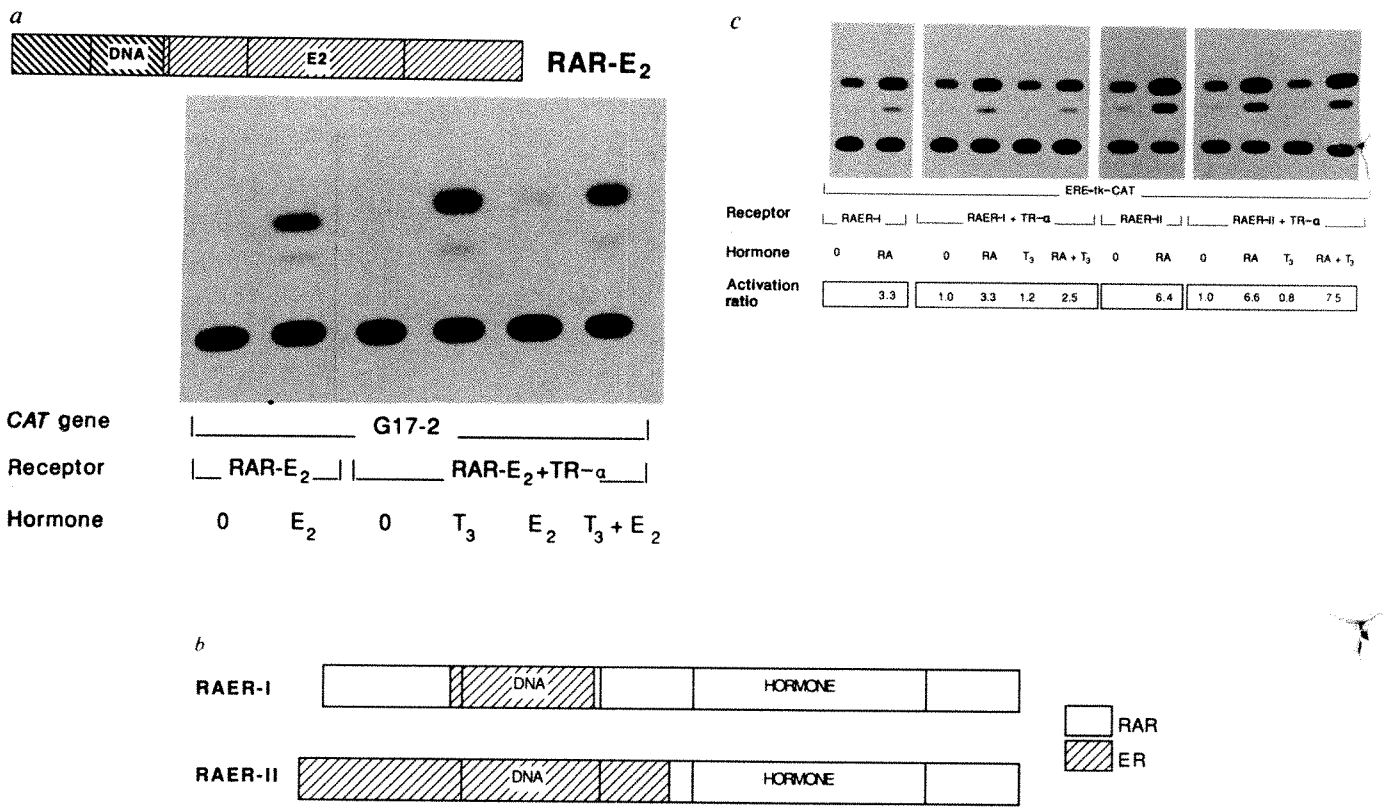

FIG. 3 Defining RAR regions sensitive to TR repression by hybrid-receptor analysis. *a*, The hybrid receptor RAR-E_2 is shown at the top. RAR-E_2 (5 μg) and TR-α (5 μg) were co-transfected with G17-2-CAT (20 μg) into F9 cells. As a control, RAR-E_2 (5 μg) and G17-2 (20 μg) were also co-transfected. Hormones were added as indicated at concentrations as above. Oestradiol (E_2) was added to a final concentration of 5×10^{-8} M. *b*, The hybrid receptor RAER-I contained the oestrogen receptor (ER) DNA-binding domain (DNA in figure) as described previously[2]. RAER-II contains amino acids 1–287 of the human oestrogen receptor, which includes the DNA-binding domain up to the hinge region, and amino acids 169–448 of RAR_ε, which includes a portion of the hinge region and the hormone-binding domain. Details of the construction to be published elsewhere (M.T. and M.P., manuscript in preparation). *c*, RAER-I and RAER-II activate transcription from an ERE–tk–CAT reporter gene in the presence of TR-α. CV-1 cells were co-transfected with PECE-RAER-I or PECE-RAER-II (2.5 μg) and PECE-TR-α (2.5 μg), together with ERE-*tk*-CAT (20 μg) reporter gene as above. The ERE-*tk*-CAT contained an ERE[12]. As a control, transfection with RAER-I and RAER-II are also shown. Transfected cells were treated with retinoic acid (RA) (6×10^{-7} M), T_3 (10^{-7} M), or retinoic acid and T_3 as indicated.

METHODS. Construction of RAR-E_2. BI-RAR (ref. 2) was digested with *Apa*I and *Hin*cII. This removes a 1-kilobase (kb) fragment with the hormone-binding domain and the terminus of the RAR-ε. A 1.15-kb *Nae*I–*Apa*I fragment of the human ER clone BI-ER (ref. 1) was ligated to the RAR-ε fragment that encodes the N-terminal and DNA-binding domains. DNA sequencing confirmed the in-frame connection between the RAR DNA-binding domain and the oestrogen receptor hinge region. For expression, a 1.8-kb *Bam*HI–*Sal*I fragment from BI-RAR-E_2 was cloned into the *Bgl*II and *Sal*I sites of PECE.

the endogenous RAR. No induction of CAT was seen in the presence of T_3, suggesting that F9 cells do not contain TRs. To investigate the retinoic-acid concentration range necessary for RAR-dependent TRE activation, we monitored CAT activity of the G17-2 construct at retinoic-acid concentrations 3×10^{-11} M–3×10^{-6} M. We observed that CAT activity, which is dependent on TREs, can be induced by RAR-ε, or F9 RAR, at retinoic-acid concentrations in the range of 0.1–1.0 nM (data not shown). These concentrations are well within the physiological range at which retinoids function[13]. To ensure that RAR-dependent TRE activation was not unique to one cell line, we repeated the same experiments using CV-1 cells. Similar results to those for the F9 cells were obtained (Fig. 1*b*).

Our data are therefore consistent with the assumption that TREs are highly efficient retinoic-acid responsive elements, suggesting that retinoic acid and T_3 modulate gene transcription, through their respective nuclear receptors, from a common family of responsive elements. This implies that certain genes in cells containing both receptors should be inducible by either T_3 or retinoic acid. To test this hypothesis and to investigate possible positive and/or negative interactions of TR and RAR, both receptors were co-transfected into the same cells, together with a TRE-CAT construct. High CAT activity was observed in the presence of both ligands (T_3 and RA). Surprisingly, no activity (or very low activity) was observed when only RA was added. These results were obtained in both F9 (Fig. 2*a*) and CV-1 cells (Fig. 2*b*), and indicate that in the presence of both receptors, TR alone controls the regulation of transcription from TREs. In the absence of T_3, TRs prevent the activation of TREs by RARs, whereas in the presence of T_3, TRs activate TREs and also allow some degree of positive cooperativity with RARs. This dominating regulatory effect can be carried out by both the two closely related thyroid-hormone receptors TR-α and TR-β, on RAR-ε (Fig. 2) and the F9 endogenous RAR. Significant repression of RAR-ε activity in F9 cells was observed with RAR-ε to TR-α ratios of between 1:1 and 4:1. Whole-cell binding assays showed, however, that RAR-ε expression is not reduced by the presence of TRs (data not shown).

To define the region(s) of RAR sensitive to repression by TRs, hybrid receptors were constructed. The hybrid RAR-E_2 receptor shown in Fig. 3*a* contains the RAR-ε DNA-binding domain and the oestrogen receptor hormone-binding domain. This receptor was able to activate a TRE-CAT gene in the presence of oestradiol (Fig. 3*a*). When co-transfected with TR-α (or TR-β), the activity of RAR-E_2 was inhibited by TRs almost as efficiently as was RAR-ε, despite the altered hormone specificity (Fig. 3*a*). The activity of the hybrid receptors RAER-I and RAER-II (Fig. 3*b*), which contain oestrogen receptor DNA-binding domains and RAR-ε hormone-binding domains, was not inhibited by TRs (Fig. 3*c*). In addition, glucocorticoid receptors did not inhibit RAR activity (data not shown). Our data, therefore, suggest that the repression of RAR activity by non-activated TRs is mediated by specific interference with the binding of RAR to DNA. Although the mechanistic details of TR repression still need to be elucidated, in the simplest model the TRs would have a dual regulatory role: in the absence of hormone they bind to TREs and function as TRE-specific repressors, preventing other related receptors from activating the TREs; in the presence of hormone they act as TRE-specific transcriptional activators.

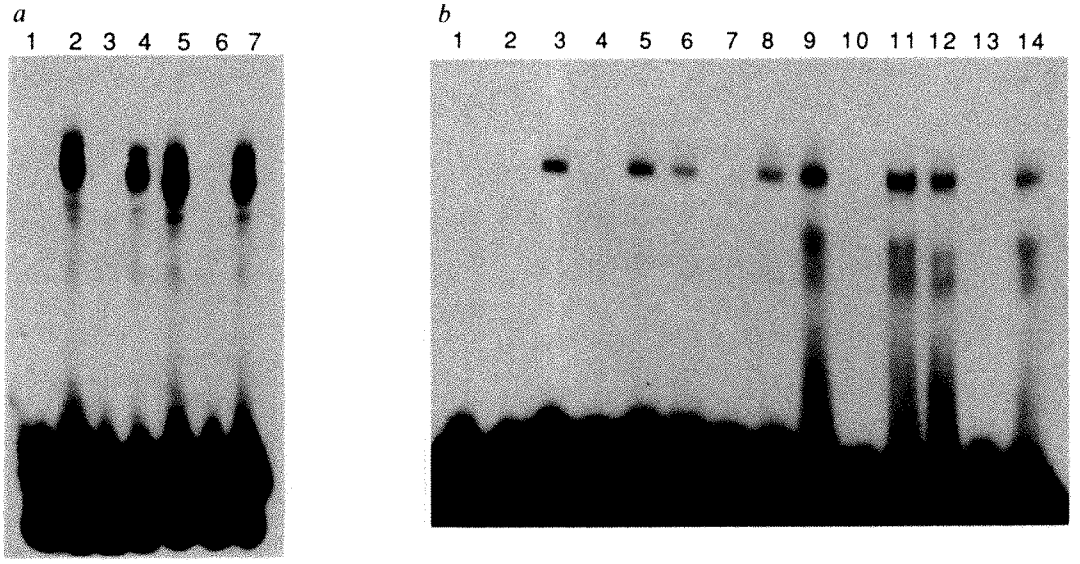

FIG. 4 DNA binding of TRs and RAR-ε *in vitro*. *a*, Thyroid-hormone receptor binds to TRE in the absence and presence of T_3. Nuclear extracts from GC cells, known to contain TR (ref. 11), were incubated with ^{32}P-labelled oligonucleotide encoding G17-2. Gel retardation analysis was used to investigate specific binding. Lane 1, migration of ^{32}P-labelled TRE; lanes 2–4, GC-cell extract in the absence of T_3; lanes 5–7, extract in the presence of T_3 (5×10^{-8} M). All extracts were incubated with ^{32}P-labelled TRE; in lanes 3 and 6, a 50-fold excess of unlabelled TRE was added; in lanes 4 and 7, a 50-fold excess of a non-specific 30-base pair (bp) oligonucleotide was added. *b*, *In vitro* synthesized RAR-ε and TR-β bind TRE in the presence and absence of ligand. Lane 1, ^{32}P-labelled TRE; lane 2, TRE plus reticulocyte lysate; lanes 3–5, *in vitro* synthesized RAR-ε in the absence of RA; lanes 6–8, RAR-ε plus retinoic acid. Lanes 9–11, *in vitro* synthesized TR-β in the presence of T_3; lanes 12–14, TR-β in the presence of T_3. In lanes 4, 7, 10 and 13, a 50-fold excess of cold TRE was added to the incubation mixture; in lanes 5, 8, 11 and 14 a 50-fold excess of non-specific 30-bp oligonucleotide was added.
METHODS. GC cells were grown in DMEM medium with 5% charcoal-treated fetal bovine serum and 10% charcoal-treated horse serum in the absence or presence of 10 nM T_3. Cell extracts were prepared in a buffer containing 20 mM HEPES, pH 7.9, 0.4 M KCl, 2 mM DTT and 20% glycerol. Aliquots with 2 μg total protein were incubated with ^{32}P-labelled G17-2 and 200 μg ml^{-1} poly(dI-dC) in a reaction mixture containing 10 mM HEPES, pH 7.9, 80 mM KCl, 1 mM DTT, 2.5 mM $MgCl_2$ and 10% glycerol. The reaction mixture was incubated at 25 °C for 20 min, then loaded on a 5% non-denaturing polyacrylamide gel containing 10 mM HEPES, pH 7.9. Electrophoresis was at 4 °C, 120 V (6.5 V cm^{-1}) for 4 h with continuous circulation of the buffer (25 mM HEPES, pH 7.9). Synthetic oligonucleotide G17-2 was radioactively labelled using the Klenow fragment of DNA polymerase I. After purification by 10% polyacrylamide gel, ~3 ng of labelled G17-2 was used for each binding assay. For competition experiments, 150 ng of cold competitor was added before addition of ^{32}P-labelled G17-2. Translated products of TR-β and RAR-ε in the presence or absence of its ligand were prepared *in vitro* as described[2]. Each binding assay used 50 mM KCl and 1 μg poly(dI-dC) per 5 μl *in vitro* synthesized protein.

We performed gel retardation experiments to determine whether TRs can bind to TREs in the absence of hormone. In one set of experiments nuclear extracts were prepared from GC cells that were known to contain TRs[11]. Extracts from cells grown in either the presence or absence of T_3 were all found to retain the labelled TREs. The specific retardation could be displaced by unlabelled TREs but not by non-specific DNA. Similar results were obtained when TR-β was synthesized by *in vitro* transcription-translation[1]. A specific protein-DNA complex was observed which was independent of the presence or absence of the hormone (Fig. 4*b*, lanes 9-14). Similar results were obtained with TR-α (data not shown). Interestingly, RAR-ε also specifically retarded the TREs in the presence or absence of hormone (Fig. 4*b*, lanes 3-8).

The results of this study demonstrate that both the human RAR-ε and the F9 endogenous RAR can mediate signal-dependent induction of transcription from TRE-containing promoters. These data are consistent with the observation that RAR-α can activate TREs from the rat growth-hormone gene[14]. Our findings, that two different types of receptors (TRs and RARs) act through the same DNA sequence, are also reminiscent of earlier discoveries that progesterone, glucocorticoid and mineralocorticoid receptors all recognize identical response elements. Pharmacologically, T_3 as well as retinoic acid are both effective in the treatment of acne[15].

The regulatory mechanism we describe here in which TRs can act as TRE-specific repressors in the absence of ligand is novel and may also be an important feature of other nuclear receptors that have been shown to bind their specific response elements in the absence of hormone[16,17]. This repression mechanism seems to be essential for the thyroid-hormone response, as isoforms of TRs that have been isolated (ref. 18, and T. Hermann, G.G., X.Z. and M.P., manuscript in preparation) seem to act as TRE-specific repressors only. A mechanism by which nuclear receptors can act as both repressors and activators specific for a particular response element would greatly increase the specificity of gene regulation. This mechanism could provide a new level of regulation by negative control for all responsive elements with overlapping receptor specificity. □

Note added in proof. While this paper was being reviewed, Damm *et al.*[23] published a similar observation on the dual regulatory role of TR-α and also showed that the v-*erb-A* protein, which is known not to bind T_3 (ref. 3), is a TRE-specific repressor.

Received 3 March; accepted 17 July 1989.

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ACKNOWLEDGEMENTS. We thank C. Glass and M. Rosenfeld for the G17-2-Luc plasmid and R. Evans for a TR-α and a TR-β clone, Michaela Pfahl for technical help and Nancy Lyman for preparing the manuscript. This work was supported by the NSF and the NIH.

Similarities between prokaryotic and eukaryotic cyclic AMP-responsive promoter elements

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ORGANISMS as diverse as bacteria and man contain genes that show transcriptional induction when the intracellular concentration of cAMP is increased[1,2]. This regulated transcriptional response is mediated through specific promoter elements located, in general, upstream from the transcription start site. In *Escherichia coli* the element responsible for cAMP-mediated transcriptional induction is the binding site for the cAMP-receptor protein (CAP). In mammalian cells the cAMP regulatory element is composed of one or more binding sites for various transcription factors[2]. In many instances the cAMP regulatory element contains binding sites for a family of proteins referred to as ATF. Here we provide evidence that some prokaryotic and mammalian cAMP-response elements are functionally related. First, we show that mammalian ATF binds specifically to some *E. coli* CAP sites, and conversely *E. coli* CAP binds specifically to some mammalian ATF sites. Second, we demonstrate that an *E. coli* CAP binding site can confer cAMP-inducibility onto a mammalian gene when assayed in transfected mammalian cells.

Both bacteria and mammalian cells contain genes that are transcriptionally activated by cAMP. The mechanism of this regulated transcription response is, however, quite different in the two systems. In bacteria, cAMP-inducible transcription is mediated through an activator protein referred to as the cAMP receptor protein (CAP or CRP). cAMP binds to CAP, converting it from an inactive form to one that can bind DNA and activate transcription[1].

In mammalian cells cAMP serves as a second messenger that transmits extracellular signals through a pathway involving the cAMP-dependent protein kinase[2]. The cAMP-responsible element in mammalian gene promoters is referred to as a cAMP regulatory element (CRE). The CRE is necessary and in some instances sufficient for cAMP-inducibility. Many mammalian CREs are composed of one or more sites for a transcription factor referred to as either ATF (refs 3, 4) or CREB (ref. 5). Recent studies have shown that ATF is not a single factor but rather a family of related proteins[6]. Therefore, the term ATF is used here to indicate a DNA-binding activity and does not imply a specific protein.

ATF is an apparently ubiquitous transcription factor: proteins with ATF DNA-binding specificity are widely distributed throughout eukaryotes[7]. An ATF consensus binding site, 5′-GTGACGT$^{AA}_{CG}$-3′, has been derived in which the 5′-TGACG-3′ motif (ATF core) is the most highly conserved portion (see ref. 4). In addition, the specific bases flanking ATF sites[8], and the context of ATF sites within a promoter[9,10] can strongly influence the transcriptional response.

The *E. coli* CAP binding site is complex, but all CAP sites contain a highly conserved portion 5′-TGTGA-3′ (reviewed in refs 1, 11). In examining a collection of *E. coli* CAP binding sites[1,11], we and others[2,12,13] recognized that in a number of instances they contained perfect or almost perfect ATF sites (Fig. 1). Also, alignment of the ATF consensus and *E. coli* CAP sites reveals that the essential guanosine contacts of the two proteins are also highly related (Fig. 1). We therefore sought to determine whether mammalian ATF sites and *E. coli* CAP sites might also be functionally related.

First, we asked whether purified mammalian ATF could in fact bind to the putative ATF sites present within some CAP sites. ^{32}P-labelled DNA probes containing the CAP sites were

prepared from the *E. coli* ARA and *E. coli* CRP promoters and incubated with either purified *E. coli* CAP or purified mammalian ATF. Binding to DNA was assayed by DNAase I footprinting (Fig. 2). As expected, purified CAP binds to the CAP site in both promoters, protecting ~25–32 base-pairs (bp) from DNAase-I digestion, and generating several DNAase-I hypersensitive sites. Purified ATF also binds in an overlapping fashion to the CAP sites, protecting ~17–19 bp centred on the 5′-TGACG-3′ motif from DNAase I digestion. This is the same extent of protection observed when purified ATF binds to authentic ATF sites in mammalian promoters[3,4] (see Fig. 3).

We then performed the reciprocal experiment, to ask whether *E. coli* CAP could bind to authentic mammalian ATF sites. Two ^{32}P-labelled DNA probes containing mammalian ATF sites were used. One of the probes was derived from the vasoactive intestinal polypeptide (VIP) promoter, a cellular cAMP-inducible promoter, and the other was prepared from the E1a-inducible adenovirus E4 promoter. Figure 3 demonstrates that ATF and CAP bind in an overlapping fashion to the ATF sites in both mammalian promoters. As expected, ATF binds quite strongly to both promoters, protecting ~20 bp of DNA from DNAase I digestion. CAP protects a region of DNA that includes the ATF site and, as observed with the *E. coli* promoters (Fig. 2), it is larger than the region protected by ATF.

These DNAase I footprinting experiments show that mammalian ATF can bind to some *E. coli* CAP sites, and that *E. coli* CAP can bind to some mammalian ATF sites. These experiments, however, do not indicate whether such binding occurs *in vivo* and if so whether it results in a regulated transcriptional response. For example in mammalian cells, sequences flanking the 5′-TGACG-3′ ATF core can significantly affect cAMP-inducibility[8]. Also, some promoters that contain consensus ATF sites are poorly inducible by cAMP (refs 9 and 10).

To address this issue, we tested whether an *E. coli* CAP site could confer cAMP-responsiveness to a mammalian gene. Transcription of the endogenous proenkaphelin (*ENK*) gene or an *ENK-CAT* fusion gene is highly inducible by cAMP (ref. 14) (Fig. 4). The promoter region responsible for cAMP-inducibility, the *ENK* CRE, functions as a regulated enhancer element[14]. When the *ENK* CRE is deleted from the *ENK-CAT* fusion gene, the basal transcription level increases slightly and the induction by cAMP decreases dramatically. Fusion of the +25 to +62 fragment of the *E. coli* CRP promoter, which contains a CAP binding site, to the truncated *ENK-CAT* fusion gene restores cAMP-responsiveness. To confirm that the cAMP-inducibility of the fusion gene was in fact due to the *E. coli* CAP site, we mutated this sequence element. The +25 to +62 fragment bearing a mutant CAP site does not restore cAMP-responsiveness to the truncated *ENK* promoter. We conclude that an *E. coli* CAP site can function as a CRE in mammalian cells.

A variety of recent studies indicate that the eukaryotic transcription machinery has been highly conserved (reviewed in ref. 15). There are also notable similarities between prokaryotic and eukaryotic transcription mechanisms. For example, both prokaryotic and eukaryotic promoters contain (A+T)-rich regions directly upstream of the transcription start site (Pribnow box in *E. coli*; TATA box in eukaryotes). Some promoter elements in

ATF Consensus:	G T G A C G T A A / C G
E. coli crp	T G T G A C G T C C
E. coli ara BAD	T G T G A C G C C G
E. coli cat site 2	T G T G A C G G A A
E. coli cat site 1	T G A G A C G T T G
E. coli gal E	T G T G A C A T G G
E. coli deo C	T G T G A T G T G T
E. coli UXU AB	T G T G A T G T G G

FIG. 1 Comparison of mammalian ATF and *E. coli* CAP sequences. The ATF consensus sequence[4] is shown in relation to seven known *E. coli* CAP binding sites. The most highly conserved portion of the CAP consensus[1,11] 5′-TGTGA-3′ is underlined. The similarity between the most highly conserved portion of the ATF consensus and the *E. coli* CAP sites are boxed. The guanosine residues protected from methylation by DMS in the ATF consensus[4] and in several *E. coli* CAP sites in which DMS protection data has been published (reviewed in ref. 1) are indicated.

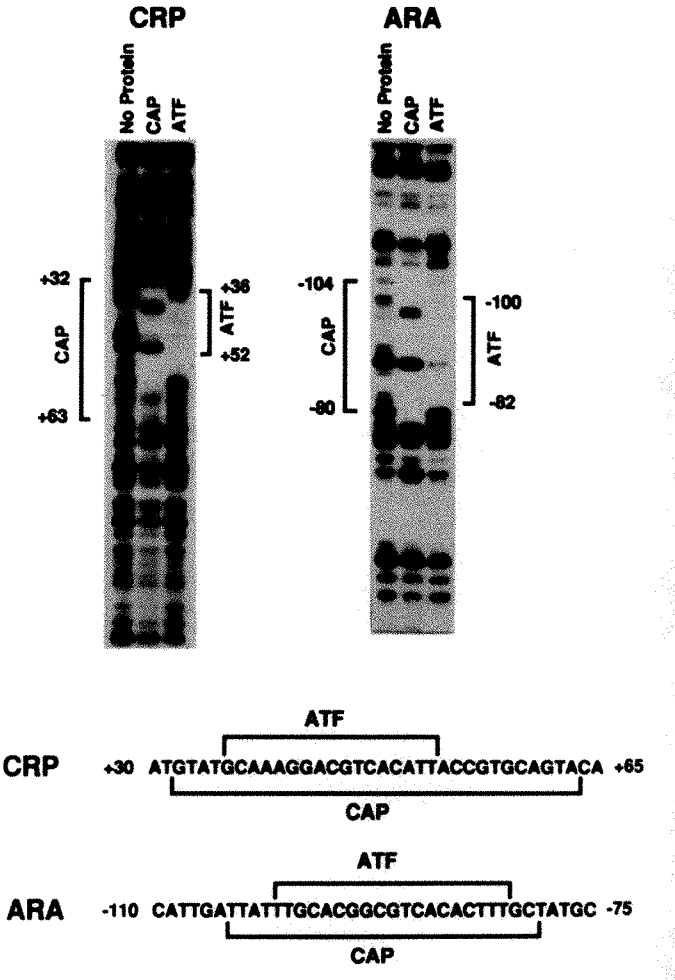
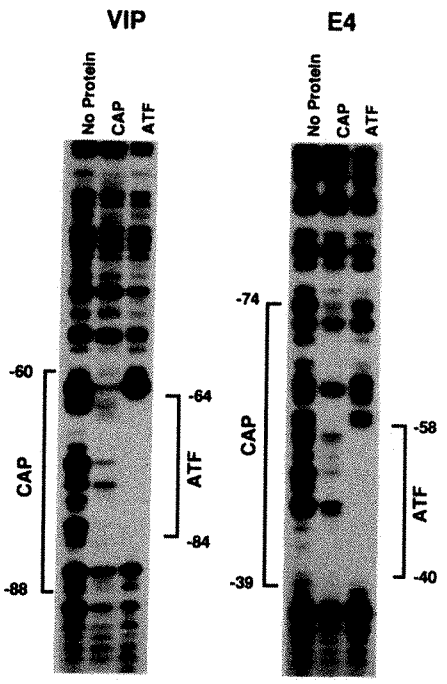


FIG. 2 Purified mammalian ATF binds to *E. coli* CAP sites. ^{32}P-end-labelled DNA probes from the *E. coli* ARA and CRP promoters were incubated with purified *E. coli* CAP protein or purified HeLa cell ATF followed by DNAase I digestion. The ^{32}P-end-labelled DNA probes used are indicated above the autoradiographs. The promoter regions protected from DNAase I digestion are indicated on the sides. The promoter sequence and regions of protection are shown below the autoradiographs.

METHODS. pARARB was constructed by inserting the *EcoRI/BamHI* fragment of pTD383 (ref. 24) (gift from R. Schleif) between the *EcoRI/BamHI* site of pGEM3. pCRPHD was constructed by inserting the *HpaI/HindIII* fragment of pHA5 (ref. 25) (gift from J. Koudelka) between the *HindIII/HincII* site of pGEM3. The probes for footprinting were the *HindIII-PvuII* fragments of pARARB and pCRPHD 3′ end-labelled at the *HindIII* site. The conditions for DNAase I footprinting of ATF were as described[4]. ATF was purified from HeLa cells as described[7]. The conditions for DNAase I footprinting of CAP were as described[26] except that 25 μg ml^{-1} of poly (dI-dC) was included and the concentrations of CAP and cAMP were 20 μg ml^{-1} and 50 μM, respectively.

both prokaryotes and eukaryotes can work when positioned at a large distance from the transcription start site (reviewed in ref. 15). *E. coli* RNA polymerase and eukaryotic RNA polymerase II are both multisubunit enzymes, and the amino-acid sequence of some of these subunits has been conserved between these organisms[16]. Finally, both prokaryotes and eukaryotes have activator proteins that function after binding to specific promoter elements. Also, there are structural similarities between some prokaryotic and eukaryotic activators both in the DNA binding domains, for example, helix-turn-helix[17]; and in the activating regions, for example, negative charge/phosphorylation[18,19] (reviewed in ref. 15).

We have shown that at least some eukaryotic ATF sites are functionally related to some prokaryotic CAP sites. Nevertheless, these elements are not identical and we do not wish to imply that all ATF sites are CAP sites, or that all CAP sites are ATF sites. It is difficult to predict how many CAP sites are also ATF binding sites, as saturating mutagenesis of an ATF binding site has not yet been performed. It seems more than coincidental, however, that there are both sequence and function similarities between promoter elements that mediate analogous transcriptional responses. Our results raise the possibility that the cAMP-responsive promoter element has been evolutionarily conserved. Consistent with this possibility is that ATF DNA binding activity is widely distributed throughout the eukaryotes[7].



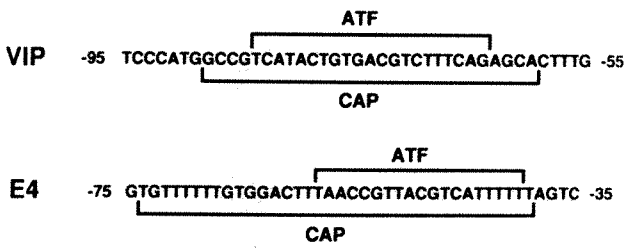


FIG. 3 Purified *E. coli* CAP binds to mammalian ATF sites. ^{32}P end-labelled DNA probes from the adenovirus E4 and VIP promoters were incubated with purified *E. coli* CAP protein or purified HeLa cell ATF followed by DNAase I digestion. The ^{32}P-end-labelled DNA probes used are indicated above the autoradiographs. The promoter regions protected from DNAase I digestion are indicated on the sides. The promoter sequence and regions of protection are shown below the autoradiographs.
METHODS. pE4BS and pVIPRN were previously described[4]. Methods as in the legend to Fig. 2, except that the probes used for footprinting were the *Hin*dIII-*Pvu*II fragments of pE4BS and pVIPRN 3′ end-labelled at the *Hin*dIII site.

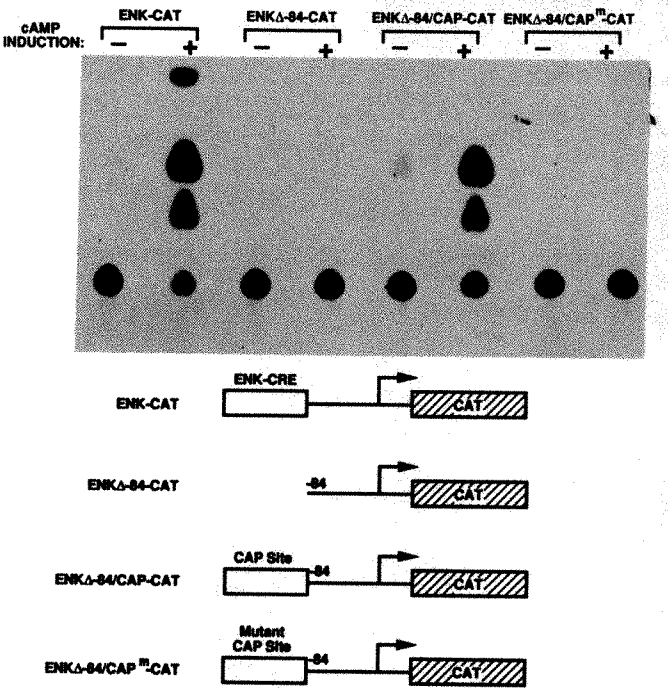


FIG. 4 An *E. coli* CAP site can confer cAMP-inducibility onto a heterologous gene in CV-1 cells. The ENK-CAT derivatives used are indicated above the autoradiograph and their structures are shown below.
METHODS. pENK-CAT and pENKΔ-84-CAT (gift from M. Comb) were previously described[14] and referred to as pENKAT-12 and pENKΔ-84, respectively. pENKΔ-84/CAP-CAT was constructed by inserting *E. coli* CRP gene sequences +25 to +62 between the *Sac*I and *Pst*I sites of pENKΔ-84-CAT. pENKΔ-84/CAP^{m}-CAT was constructed by inserting between the *Sac*I and *Pst*I sites a double-stranded synthetic oligonucleotide containing *E. coli* CRP gene sequences from +25 to +62, except that the CAP site at position +50 to +46 was mutated from 5′-TGTGA-3′ to 5′-TGGAC-3′. Transient transfection assays were performed in CV-1 cells. The transfection, cAMP-induction and CAT assays were performed as described by Comb *et al.*[14] except that cAMP-induction was performed with 25 μm Forskolin and 0.5 mM IMX.

We have identified promoters containing overlapping consensus ATF and *E. coli* CAP sites in a wide variety of eukaryotes (Table 1). The cAMP-inducible human VIP promoter (−78 **TGTGACGTCTTTCAGA** −63) and the mouse Aα promoter (−131 **TGTGACGTCATTCACA**) −116) contain CAP sites with

TABLE 1 CAP/ATF overlap in eukaryotic promoters

Organism	Promoter	Sequence	Position
		CAP	
Human	VIP	TGTGACGTCT	−78/−69
	BSF2/IL-6	TGTGACGTCC	−218/−227
	apolipoprotein CII	TGTGACGTGA	−169/−160
Adenovirus	E1a	TGTGACGTGG	−437/−428
	E3	TGTGACGAAA	−53/−62
	E4	TGTGACGTGG	−268/−259
Mouse	Aα	TGTGACGTCA	−131/−122
	NGF	TGTGACGAGC	−72/−63
Chicken	U4	TGTGACGTAG	−57/−48
S. cerevesiae	PGK	TGTGACGAAA	−416/−425
	CDC4	TGTGACGTTT	−222/−231
		ATF	

The table shows the partial sequences of 11 eukaryotic promoters that contain overlapping consensus ATF and CAP binding sites identified in a random literature search. The CAP and ATF binding elements are shown by brackets. Primary refs for promoter sequences available on request.

dyad symmetry, which match the consensus better than any natural *E. coli* CAP site (reviewed in ref. 1).

The possibility that a regulatory promoter element has been evolutionarily conserved is not without precedent. Genetic and physical evidence indicate that phage 16-3 of the nitrogen-fixing bacterium *Rhizobium meliloti* is unrelated to the lamboid phage 434. Sequence comparisons and functional studies, however, have shown that some operators of these two phages are similar[20]. Another example is the eukaryotic heat-shock element, which is identical in all eukaryotes. Interestingly, the heat-shock transcription factors, which recognize heat-shock elements, are regulated differently in higher and lower eukaryotes. The heat-shock factors may be less conserved than the regulatory promoter elements[21-23]. □

Received 13 June; accepted 17 July 1989.

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ACKNOWLEDGEMENTS. We thank Dr Joe Krakow for purified *E. coli* CAP protein, Drs R. Schleif and J. Koudelka for gifts of plasmids, Dr M. Comb for plasmids and discussions and Drs K. Martin and R. Bushman for critical comments. This work was supported by grants from the NIH and The Chicago Community Trust/Searle Scholars Program to M.R.G.

Detergent structure in crystals of a bacterial photosynthetic reaction centre

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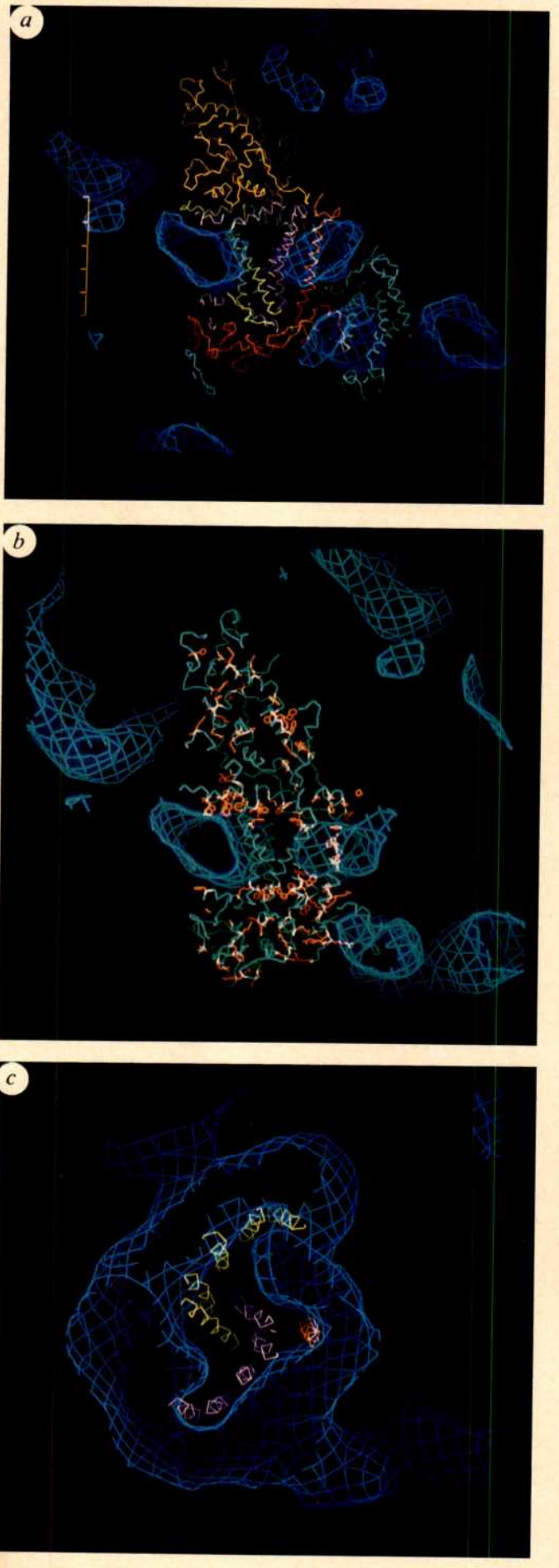
RECENT evidence shows that membrane-bound proteins can be crystallized successfully in the presence of detergent[1-3], which seems to facilitate the ordered packing of the proteins by binding to their hydrophobic surfaces in micellar manner[4,5]. This approach has enabled the molecular structures of two bacterial photosynthetic reaction centres to be solved at high resolution by X-ray crystallography[6-9], each of which has provided insights into the mechanism of photo-activated electron transport across the cell membrane. The detergent, however, although present in high concentration in the crystals, is not seen in these high-resolution structures because of disordering. To determine the structural motifs formed by the detergent that are involved in crystal packing, we have therefore generated a low-resolution structure using neutron diffraction with contrast variation. We find that the detergent is concentrated in rings which fill all the available space around the membrane-spanning α-helices of the reaction-centre protein subunits L, M and H. These rings are interconnected throughout the crystal lattice by short cylindrical detergent bridges such that zig-zag chains are formed parallel to the *c* direction. The average structure of the detergent therefore is spatially complementary to the structure of the reaction-centre complex and provides a model for the interaction between the lipid bilayer and the complex *in vivo*.

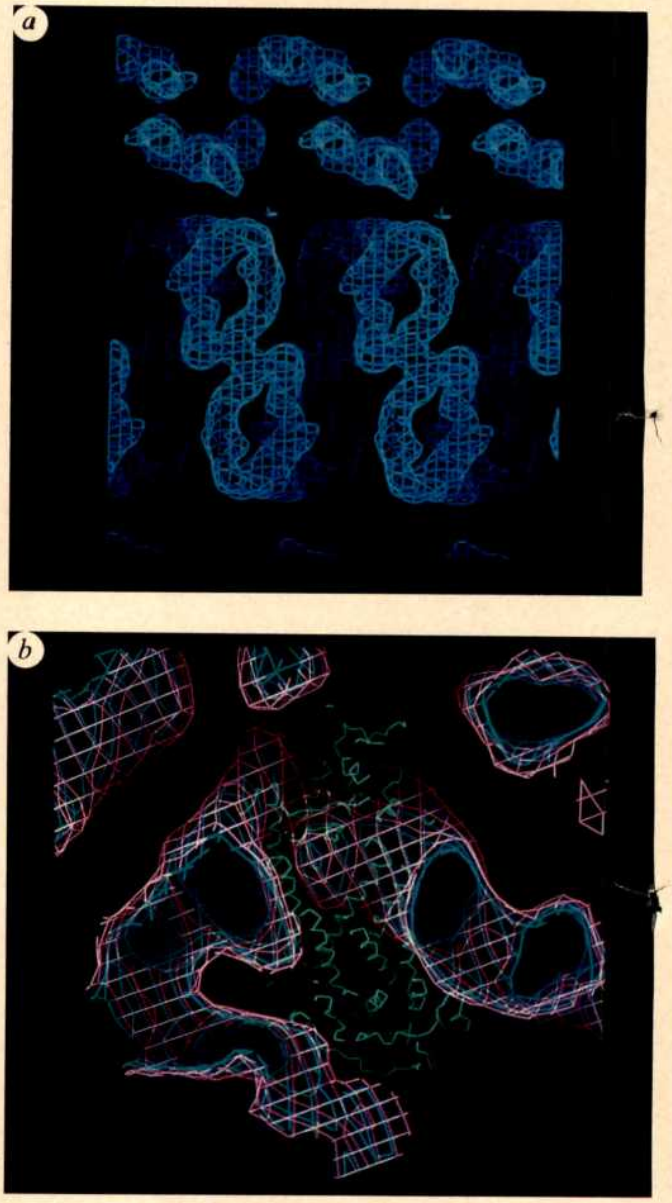
Because of disorder of the detergent in the reaction centre crystal (only one detergent molecule could be localized in the X-ray diffraction analysis) (J.D. *et al.*, in preparation), its structure could only be determined as a molecular average at low resolution. To analyse the structure we used low resolution neutron diffraction with H_2O/D_2O contrast variation[10]. This consists of diffraction measurements from several reaction centre crystals, differing in their H_2O/D_2O content and thus in the relative contrast of the reaction centre molecule and the detergent with respect to the solvent.

The main quantitative information contained in contrast variation data is the variation of the phase of the structure factors as a function of contrast[11]. The method is most useful in the case of a partially known structure. The phases of the complete structure at any contrast can be derived from the phases of the known part of the structure at a contrast where the scattering density of the unknown part is matched out by the solvent density. Details of the phasing method are described elsewhere[12]. The result of the calculation is a statistical best estimate of the structure factors in any contrast, from which the density maps are then calculated. Results are summarized in Table 1.

The detergent is most clearly seen at a contrast close to 40% D_2O where the average protein density is matched out. At this contrast, the detergent has a relatively large negative contrast and its structure can be seen using an appropriate contouring level. The most striking feature we found is that the detergent appears as a distinct phase in the aqueous solvent region, forming a micelle around the protein, very much like the model proposed from small angle X-ray scattering data by Sardet *et al.*[13] for the rhodopsin-*N,N* dimethyldodecylamine *N*-oxide (LDAO) complex. A reaction centre molecule from *Rhodopseudomonas viridis* consists of (1) a cytochrome *c* subunit at the periplasmic side of the membrane *in vivo* (at the top in Fig. 1); (2) a large H subunit at the cytoplasmic side *in vivo* (at the bottom in Fig. 1); and (3) in between, two subunits L and M, each forming five long, almost parallel, membrane-spanning α-helices[7]. These are connected by segments in contact with the cytochrome and the H subunit respectively, which form a surface parallel to the membrane plane and perpendicular to the helices (the limiting surfaces, Fig. 1*a* and *b*). The bulk of the detergent phase appears concentrated in rings around each reaction centre molecule at the level of the membrane-spanning α-helices of L, M, and H. The average plane of each ring is perpendicular to the approximate 2-fold axis relating the trans-membrane α-helices of subunits L and M. Another protein-detergent contact is found between the H subunit in the region H48-H55, which is poorly defined in the X-ray map, and the detergent ring of a neighbouring reaction centre molecule. An ordered LDAO molecule was found in the refined X-ray map (J.D. *et al.*, in preparation); its aliphatic chain lies well within the detergent density and its polar head outside.



◀ FIG. 1



▲ FIG. 2

FIG. 1 View of the reaction centre molecule surrounded by detergent (in blue); subunit H, orange; subunit M, mauve; subunit L, green; cytochrome, yellow; pale blue: parts of neighbouring molecules. The scale bar is marked every 10 Å. *a*, Only the Cα backbone is shown. The transmembrane α-helices are around 40 Å long, but as none of them is perpendicular to the detergent ring, the average distance between the 'limiting surfaces' is 25–30 Å. The detergent ring cross-section is smaller in the region of the H transmembrane α-helix, where it is limited by hydrophilic regions of the neighbouring reaction centre molecule. *b*, The protein surface is rich in Trp, Arg and His residues, especially on the 'limiting surfaces', where we observe these side-chains in intimate contact with the detergent surface. The reaction centre Cα backbone is in green, with the relevant side-chains in red. *c*, A cross-section through the reaction centre at the median level of the detergent ring showing the α-helices surrounded by the detergent; colours as in *a*.

FIG. 2 The packing of the reaction centre molecules in the $P4_32_12$ lattice can be described as a helical stacking of pairs of reaction centre molecules in two rows per unit cell parallel to [0, 0, 1]. One of these rows is centred at (0, 0, 0) and the other at (1/2, 1/2, 0), related by 4_3 symmetry. The detergent rings of the molecules within a single row are connected two by two through short cylindrical detergent contacts, or 'bridges', with a similar cross-section as the rings themselves. These bridges are located on crystallographic twofold axes and are of two types, a straight one and a hairpin one. The detergent is thus distributed in parallel zig-zag chains of interconnected rings throughout the crystal. These chains, parallel to the *c* axis, are only very loosely connected between them through weak detergent density found in the vicinity of the 4_3 crystal axes. *a*, View (down **110**) of several unit cells showing the packing of the detergent rings within the crystal lattice, showing a zig-zag chain along the 2_1 axis parallel to *c*. *b*, View of one detergent ring, with the transmembrane helices of L, M and H subunits shown schematically in green, connected through one straight (at the right) and one hairpin (at the left) bridge to neighbouring rings. Two contour levels are shown: at σ (blue) and at 0.5 σ (mauve), where σ is the r.m.s. of the neutron scattering length density, demonstrating that the cross-section of the detergent ring is fairly insensitive to the choice of contouring level and that the bridges are probably not artefacts.

The detergent ring thickness perpendicular to the membrane plane is about 25–30 Å, which is close to the value given for LDAO micelles in pure water[13] and is of the order of twice the length of an extended LDAO molecule (15–16 Å). This suggests that the LDAO molecules in the immediate neighbourhood of the protein are oriented parallel to the protein as in a lipid bilayer. It is remarkable that, all around the reaction centre molecule, the detergent ring fills the entire space available between the limiting surfaces of subunits L and M. This could indicate that (as much as the detergent-protein interaction is a valid model for membrane-protein interaction) in the native membrane the contact surface between the transmembrane helices and the membrane is only 25–30 Å wide, ending on either side at the limiting surfaces formed by the more hydrophilic parts of L and M. If the membrane is ≥40 Å thick on average[14], it will have to rearrange to fit into the available space around the transmembrane helices of the reaction centre structure, in a more complicated way than the simple cylindrical model proposed by Yeates *et al.*[15]. In the native photosynthetic membrane the reaction centre is surrounded by light-harvesting complexes[16,17], whose interaction with the reaction centre protein may in fact be represented by some of the protein-detergent contacts in the present crystal structure.

The detergent rings are interconnected throughout the crystal as shown in Fig. 2. This network constitutes a three-dimensional ordered structure of a liquid-state phase, which is reminiscent of the structure of amphiphilic liquid crystalline phases. We have here a three-phase system where the three interfaces (detergent-protein, detergent-solvent, solvent-protein) are of similar extent and play an important role in the energy of the system. The detergent as defined by the contouring level shown in Fig. 1, occupies 13–14% of the unit cell volume. With a volume of 480 Å (ref. 3) per LDAO molecule, this would correspond to a concentration of the order of 0.5 M for the detergent in the crystal. With respect to molecular packing in the lattice, it is clear that the protein–protein interaction between the cytochromes and the H-subunits is important. The regions of protein–protein contact seem free of detergent density, although the detergent ring is touching one of the H-H contact regions. The detergent nevertheless plays a decisive role in the crystal packing. It prevents aggregation of the exposed apolar surfaces of the reaction center molecules in general and, more specifically, interacts tightly and stereo-specifically with the protein. This property may explain the pronounced dependence of membrane protein crystallization on the nature of the detergent used. □

TABLE 1 Summary of data and statistical results of phasing and modelization

Data:				
Contrast measured (% D_2O)	0%	15%	55%	87%
R_{sym} (550 unique reflections)	0.036	0.078	0.037	0.055
Structure determination:				
Contrast used for phasing (% D_2O)	0%	40%		87%
Initial *R*-factors ($F^{(1)}_{calc}$ versus F_{obs})	0.19	0.64		0.64
Final *R*-factors (F_{best} versus F_{obs})	0.05	0.23		0.27
Average phase change (deg)	10.7	51.4		61.9
FOM after first F_{best} calculation	0.808	0.714		0.833
Refinement:				
Initial *R*-factors ($F^{(2)}_{calc}$ versus F_{obs})	0.19	0.36		0.46
FOM after iteration of F_{best} calculation	0.834	0.764		0.861

Crystals of the reaction centre from *Rps viridis* were grown as described earlier[3] (space-group $P4_32_12$ with $a=b=223.5$ Å and $c=113.6$ Å) in 2.5 M ammonium sulphate. The crystals are parallelepipeds typically $0.4\times0.4\times0.8$ mm^3 in size, the long dimension corresponding to the *c*-axis. The diffraction experiments were performed using crystals with four different contrasts, obtained by soaking them in a buffer of the same chemical composition as the mother liquour, but with a different D_2O/H_2O concentration (0, 15, 55, and 87% D_2O). The detergent used in these crystals was LDAO whose average scattering density is very close to that of H_2O containing 2.5 M ammonium sulphate. The concentration of zero contrast between solvent and detergent is thus very close to 0% D_2O, while at 40% D_2O the protein density is matched by the solvent. Neutron diffraction experiments were carried out on D17 and DB21, at the Institute Laue-Langevin (ILL), Grenoble (France). Both instruments use fairly long wavelength neutrons (11 Å and 7.5 Å respectively) and two-dimensional flat position sensitive detectors (BF_3 gas detector[18] and Anger camera scintillation detector[19] respectively). The data collection was made using stationary step scans with 0.15 deg between steps (typically 10 min per step). The data reduction was performed using a chain of programmes developed at ILL (refs 20, 21). R_{sym} is defined as $\Sigma\,(I-\langle I\rangle)/\Sigma I$, where I is the intensity of an individual reflection, $\langle I\rangle$ the average over symmetry equivalent reflections, the summation being over all reflections. Phasing: $F^{(1)}_{calc}$ corresponds to the known X-ray structure. As expected, the agreement is best at 0% D_2O contrast where the detergent is matched out. No adjustment or refinement of the protein model with respect to the measured data was performed, other than the calculation of resolution independent scale factors, one per data set. F_{best} is the modulus of the least-squares (best) estimator of the true **F**, resulting from the statistical combination of the F_{obs} and $F^{(1)}_{calc}$ probability distribution[12]. The figure of merit (FOM) is the average of the cosine of the difference between the measured and calculated phase differences between the contrast under consideration and a reference contrast. $F^{(2)}_{calc}$ corresponds to the X-ray structure plus a detergent model deduced from the first 40% D_2O density maps. A marked improvement is seen for the contrasts where the detergent is not matched out, demonstrating the validity of this model. *R*-factors are calculated for reflections with $F_{obs}>2\sigma(F_{obs})$.

Received 20 March; accepted 23 June 1989.

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CORRECTIONS

The FCγ receptor of natural killer cells is a phospholipid-linked membrane protein

David Simmons & Brian Seed

Nature **333,** 568–570 (1988).

THIS letter reported the isolation and partial characterization of a cDNA clone encoding a phospholipid-anchored form of CD16/FcRγIII. We regret that the data purporting to show displacement of monomeric IgG1 cannot be reproduced, nor can the data showing phospholipid anchorage of the CD16 form found on peripheral-blood mononuclear cells that do not adhere to nylon wool. In addition recent studies have shown that there are at least two forms of CD16: a phospholipid-linked form expressed by granulocytes and a conventionally anchored form expressed by natural killer cells (L. Lanier *et al. J. Immun.* **141,** 3478–3485; 1988; B. J. Scallon *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 5079–5083; 1989; J. Ravetch & B. Perussia, personal communication).

The sequence of the cDNA clone, and its attachment to the cell surface by a phospholipid anchor, have been confirmed by further investigation. Jeffrey Ravetch, however, has kindly pointed out that the molecular weight marker scale in Fig. 2*b* gives inappropriately low values for the CD16 genomic DNA fragment lengths.

We would like to sincerely apologize for the confusion and misdirected effort these errors have provoked. □

Expression of a large family of POU-domain regulatory genes in mammalian brain development

Xi He, Maurice N. Treacy, Donna M. Simmons, Holly A. Ingraham, Larry W. Swanson & Michael G. Rosenfeld

Nature **340,** 35–42 (1989).

IN this Article there is an error in the amino acid sequence encoded by Tst-1 gene (hybridizing to 7.6, 4.4, 3.2 and 2.4 Kb brain mRNAs) reported in Fig. 1*b*, two residues before the end of POU-specific domain. The correct sequence is ...WLEETD... instead ...WLEEAD.... □

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The present director General will retire in April 1991 and the Board of Trustees has begun an international search for his successor. The Board plans to identify the successful candidate by September, 1990, and the appointment will commence early in 1991.

The new Director General of CIP will possess a proven record in management and administration; extensive experience and high qualifications in agricultural research; a sound knowledge of and experience with agriculture in developing countries; vision and leadership qualities and an ability to communicate and inspire high performance from scientists and administrators of different nationalities and disciplines.

A competitive salary with fringe benefits will be offered. Requests for further information should be directed to the **Chairman of the Search Committee, David L. Call, Dean, College of Agriculture and Life Sciences, Cornell University, Roberts Hall, Ithaca, New York 14853, USA. (FAX 607-255-0788).**

Applications for the position should be forwarded (preferably before October 20, 1989) to:

CIP Search Committee, c/o Dr. J. W. Meagher
3 Kingfield Court, Burwood, Vic. 3125
AUSTRALIA
FAX: 61 3 885159
Telex: AA 30625
Attention ME 3715

(NW4072)A

RESEARCH FELLOW A$31,525-A$38,567

Division of Plant Industry, Canberra, Australia

THE PROJECT: The project will form part of a long term work-program on plant performance in relation to global change in atmospheric composition. It will also be an input to the newly formed CSIRO Greenhouse Effect/ Climate Change Program emphasising regional climate change prediction. The project will be paralleled by field investigations and biophysical modelling of evapotranspiration at different spatial scales as a function of environmental and genetic variables.

THE JOB: The appointee will undertake research into the effect of atmospheric carbon dioxide concentration on stomatal conductance, leaf area development, transpiration from plant canopies and water use efficiency. Emphasis will be on characterising and quantifying the interaction between soil water content and atmospheric carbon dioxide in regulating evaporation from vegetation under defined temperature and humidity conditions.

THE PERSON: Applicants should hold a Ph.D. degree or equivalent qualification with research achievement in plant physiology, plant water relations and productivity. Experience with leaf and whole plant gas exchange analyses and with computer programming and data logging is required.

CONDITIONS: Appointment will be for a period of 3 years with Australian Government Superannuation benefits available.

MORE INFORMATION: Prospective applicants are invited to telephone Dr Roger M. Gifford on (062) 46 5441 (or from outside Australia on 61 62 46 5441 (09:00-17:00) or on 61 62 47 5126 after office hours) for further information. A copy of the detailed duty statement and selection criteria may be obtained by contacting tel. 61 62 46 5206 or fax 61 62 47 3785, or telex Australia 62351.

APPLICATONS: Applications should be submitted by 24 September 1989 quoting reference number PGN8911. They should be framed against the selection criteria, and state relevant personal particulars including details of qualification, experience and publications. Applicants should give details of at least two referees, and address their applications to:

The Chief
CSIRO
Division of Plant Industry
GPO Box 1600
CANBERRA, 2601 AUSTRALIA

CSIRO AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W6434)A

ABI-PLN 89/11

UNIVERSITY OF ABERDEEN
PROSAMO
POSTDOCTORAL RESEARCH ASSISTANT IN MOLECULAR BIOLOGY and ENVIRONMENTAL BIOTECHNOLOGY

PROSAMO is a major new interdisciplinary DTI/industry/AFRC funded three year project to develop methods that will provide the basis for assessment of the risks arising from the release of genetically manipulated plants and micro-organisms into the environment. The microbial programme will require the co-ordinated activities of a team of scientists at both Aberdeen and Essex Universities.

Applications are invited for a Postdoctoral Research Assistant with experience in molecular biology to work on the cloning and manipulation of *lux* genes and the construction of luminescent soil bateria for the subsequent development by other workers within the group of methods to extract and trace very small numbers of such bacteria in laboratory microcosms and field samples. The successful applicant will be a member of a large group with 2 other PDRA, assisted by 2 PGRA.

Salary within the range £10,458-£12,381.

Further particulars and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** (tel 0224 273500) to whom applications (2 copies) should be returned by 8 September 1989 quoting reference number LW/016. (1375)A

ASSISTANT PROFESSOR OF TROPICAL PUBLIC HEALTH

Applicants are sought for the position of Assistant Professor of Tropical Public Health. The applicant should be a molecular biologist with experience in developing diagnostic DNA probes in infectious diseases, preferably tropical diseases, and their testing and application in the field. The applicant should be committed to developing a research program in this area, and would be expected to participate in several research and training projects outside the U.S. which involve the use of DNA probes and the polymerase chain reaction. The applicant would also be expected to participate in the teaching program of the department. Women and minority candidates are encouraged to apply.

Applicants should send their CV, a brief statement of their future research interests, and should arrange to have three letters of recommendation sent. Please send materials to:

Search Committee for Assistant Professor (Diagnosis)
Department of Tropical Public Health
Harvard School of Public Health
665 Huntington Avenue
Boston, MA 02115

(NW4086)A

RESEARCH FELLOW

(Satellite Antenna Development)

$A31,525-$A45,699

CSIRO DIVISION OF RADIOPHYSICS
MARSFIELD, NSW, AUSTRALIA

As a research leader with a commitment to the needs of the information and communications industries, the Division of Radiophysics is engaged in research, development and consulting for signal and image processing, VLSI design, Gallium Arsenide device design, millimetre-wave and microwave receiver technology, earth stations and satellite antennas.

The Division is seeking a scientist or engineer to participate in the continuing development of sophisticated satellite antenna technology. You will join a group acknowledged as being Australia's foremost centre of expertise in this area. You will be expected to contribute not only to the development of new analytical and experimental techniques, but also to have a major involvement in contract research with Australian and overseas firms, a major component of the group's research activity.

You will participate in one or more of the following project areas:

- Optimum design of shaped beam and reconfigurable beam antennas
- Multiple-access earth station antennas
- Corporate feed networks for on-board spacecraft antennas
- Near-field antenna measurements

You will need to possess the following qualifications:

- a PhD, or equivalent qualifications, in physics or electrical engineering
- good mathematical skills
- experience in microwave analytical and/or experimental techniques
- preferably some experience with industrial projects

The appointment will be for a period of 4 years and will offer Australian Government superannuation benefits. Please contact Dr Geoff Poulton telephone (02) 868 0222, or fax 010+61+2+8680457, for further information.

Applications should quote reference number A4977 and be submitted by 25 September, 1989. They should include the names of at least two professional referees and be addressed to:
The Chief,
CSIRO Division of Radiophysics,
PO Box 76, Epping,
NSW 2121, Australia

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

(W6433)A

UNIVERSITY OF LEICESTER
DEPARTMENT OF BOTANY

POST-DOCTORAL RESEARCH ASSISTANT & TECHNICIAN IN PLANT MOLECULAR BIOLOGY

Applications are invited for the above posts to join an established team investigating gene expression and protein secretion at wound sites in monocots and dicots. The project will involve part of the following themes:

(1) Characterisation of the promoter region of a recently-isolated wound site specific gene from asparagus in transgenic plants of several species.
(2) Clone genes coding for proteins secreted from mechanically-isolated cells of several species after wounding.
(3) Design constructs to allow the expression and secretion anti-bacterial proteins in transgenic plants.

Suitable applicants should have experience in recombinant DNA technology and/or molecular biology. The appointments are tenable for 3 years starting on 1st October 1989 (or soon thereafter) and are on the Research Associate IA scale £10,458-£16,665) and the Technician Grade A scale (£6,040-£6,939), according to qualifications and experience.

Informal enquiries and formal applications, including C.V. and names of two referees as soon as possible to **Dr John Draper or Dr Garry Whitelam, Botany Department, University of Leicester, Leicester, LE1 7RH, U.K.** Tel. (0533) 523392. (1398)A

POSTDOCTORAL POSITION METABOLISM

CEDARS-SINAI/HARBOR-UCLA MEDICAL CENTERS

Research in intermediate metabolism *in vivo* and *in vitro*, with major interest in regulation of carbohydrate metabolism, application of radioactive and heavy tracers and the use of mass spectroscopy. Position available immediately, funded for up to 3 years, salary range: $21K-$26K/year. Qualified applicants write with CV and refs to **Dr. Joseph Katz, Cedars-Sinai/Harbor Medical Centers, REI, A-17 Annex, 1000 W. Carson St., Torrance, CA 90509, USA.** (NW4093)A

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA

SCIENTIFIC OFFICER (BAND II)
2 years appointment

MYCOLOGY AND BACTERIOLOGY DEPARTMENT

Applications are invited for the above post available from 10 October 1989 for a two year period to assist in work on the development of serological methods to determine contamination of potato seed stocks by *Erwinia carotovora*.

Latent infection of potato seed tubers by the blackleg pathogen, *Erwinia carotovora* sub sp. *atroseptica* (Eca), is widespread and cannot be detected by seed certification. Of the several factors affecting disease development seed contamination level is crucial. The objective of the project is to develop a serological user-friendly method to quantify seed stock contamination by Eca. This would involve the preparation of Eca specific polyclonal sera using whole cells and erwinia LPS as antigens, absorption of cross reacting antibodies against other erwinias and contaminating bacteria, development of ELISA assays and other serological detection and enumeration methods: development of a stock sampling procedure, and determination of the threshold level of seed contamination for blackleg development of different cultivars.

Salary: £8,574–£10,994

Non-contributory superannuation.

Qualifications: Degree in a relevant subject or HNC with relevant post-qualifying experience in Biology/Microbiology, experience in serology is desirable.

The Institute is an equal opportunities employer.

Curriculum vitae complete with the names and addresses of three referees should be sent to the Personnel Officer, by Friday 8th September 1989 quoting the appropriate reference (Ref. MB/4/89).

(1407)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PHARMACOLOGY

2 Post-doctoral Research Associates

Applications are invited for the above positions to work on the following projects:

(1) The role of potassium channels in the control of ventromedial hypothalamic neurone firing in response to changes in extracellular glucose concentrations and the effects of potassium channel inhibitors. This project is funded by a BDA project grant (to Dr M L J Ashford) and the starting salary is up to £13,527. The appointment will be for two years initially.
(2) Pharmacological modulation of potassium channel activity and the control of human myometrial excitability. This project is funded by an MRC project grant (to Dr M L J Ashford and Professor S K Smith (Department of Obstetrics and Gynaecology)). The appointment will be for three years and the starting salary is up to £12,381.

Experience in electrophysiology (patch clamping) is desirable for both positions. Applications with CV and names of two scientific referees should be sent, by 29 September, to **Dr M L J Ashford, University of Cambridge, Department of Pharmacology, Tennis Court Road, Cambridge CB2 1QJ.** Informal enquiries telephone Dr Ashford on (0223) 334000 or FAX (0223) 334040. (1396)A

UNIVERSITY OF EDINBURGH
PLANT MOLECULAR BIOLOGY

POSTDOCTORAL RESEARCH SCIENTIST

Applications are invited from suitably qualified and enterprising Postdoctoral Research Scientists for an exciting new project aimed at manipulating the synthesis of calmodulin in potato by genetic transformation with the calmodulin gene. The position is funded by the Agricultural and Food Research Council for three years, and involves collaboration with the Scottish Crop Research Institute. Informal enquiries and applications, together with names of two referees, should be made to **Drs. A. J. Trewavas and S. M. Smith, Department of Botany, University of Edinburgh, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH. U.K.** (Tel. 031-667-1081, ext. 3305). *Please quote reference no. 5696.* (1383)A

The Research Institute of Molecular Pathology in Vienna (I.M.P.), a joint venture of Boehringer (Ingelheim) and Genentech (South San Francisco) was established as a center of scientific research excellence in the life sciences, with a focus on the study of the molecular basis of disease. The I.M.P. is dedicated to basic research and to the advancement of Science.

In the longer term it is intended that basic scientific concepts developed at this new center will spawn identification of potentially useful products in therapeutic and diagnostic areas for commercial development within Genentech and Boehringer. A special asset of the I.M.P. is its access to technical know-how and facilities in the mother companies.

In defining and achieving its strategic research goals, the I.M.P. has recourse to a scientific advisory board which is composed of preeminent scientists in the field of oncogenesis, cell biology and developmental biology.

The I.M.P. is seeking

Junior & Senior Scientists

with leader qualities and a record of research excellence in the area of molecular genetics, cell biology and growth control. Both PhDs and medical doctors with appropriate research experience are encouraged to apply.

Applicants should enclose a curriculum vitae, a list of publications, and a one page summary of their future research interests.

Applications are to be sent to: **Prof. M.L. Birnstiel, Research Institute of Molecular Pathology (I.M.P.), Dr. Bohr-Gasse 7, A-1030 Vienna, Austria, fax no.(222)78 93 90.** (W6427)A

Postdoctoral appointments in

UEA NORWICH

Synthetic Organic Chemistry and Enzymology/Cell Biology

Applications are invited for the above research posts, funded by the **MRC AIDS Directed Programme,** to work on a collaborative project in the Schools of Chemical and Biological Sciences. The project involves the design, and synthesis of novel glucosidase II inhibitors and their biological screening, particularly with respect to their ability to inhibit the production of specific glycoproteins. Promising compounds will be submitted to one of the MRC testing centres for HIV screening.

POST 1: To work with Dr. R. J. K. Taylor and Dr. A. H. Haines on the design and synthesis of glucosidase inhibitors. Research experience in synthetic organic chemistry is essential.

POST 2: To work with Dr. A. P. Dawson and Dr. I. Gibson on biological screening studies. Research experience in enzymology and/or cell biology is essential.

The appointments are for three years commencing October 1989. The salaries are on the RA 1A scale. £10,458-£16,665 p.a., at an appropriate point.

Applications (enclosing C-V and the names of 2 professional referees) and enquiries to:

***POST 1:* Dr. R. J. K. Taylor, School of Chemical Sciences, UEA, Norwich NR4 7TJ (0603-56161 ext. 2044).**

***POST 2:* Dr. A. P. Dawson, School of Biological Sciences, UEA, Norwich NR4 7TJ (0603-56161 ext. 2760).** (1370)A

THE UNIVERSITY OF BIRMINGHAM SCHOOL OF BIOLOGICAL SCIENCES LECTURESHIP IN MICROBIAL MOLECULAR GENETICS

A position is available for an active research scientist in the Microbial Molecular Genetics and Cell Biology Group of the School of Biological Sciences (the former Departments of Genetics, Microbiology, Plant Biology and Zoology). The lectureship is associated with the appointment of Professor Nigel Brown to the Chair of Molecular Genetics and Microbiology.

Preference will be given to applicants with experience in the molecular biology of bacterial or other microbial systems. The overriding criterion will be academic excellence. The successful applicant will be expected to develop an independent research programme and to participate in teaching. Opportunities will exist to collaborate with other members of the School. The appointment will be on the Lecturer Scale, £10458-20469 plus superannuation.

For an informal discussion of the position please contact Professor N.L. Brown (Tel 021-414-6556, Fax 021-414-5925). For further particulars and an application form telephone 021-414-6483 (24 hour answerphone) quoting reference BS/2004. Applications (3 copies) should be sent to the **Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham, B15 2TT** by 20th October.

AN EQUAL OPPORTUNITIES EMPLOYER (1388)A

University of Cambridge Departments of Chemistry and Physics

A position is open for an **organic chemist as a graduate assistant** in an active research group working in the general area of molecular electronics. The pursued programme involves collaboration between the Departments of Chemistry and Physics with the successful applicant having the unique opportunity to work in both Departments. Only applicants who are graduates (or equivalent) and have experience in techniques used for synthetic organic chemistry need apply. The successful applicant will participate in the synthesis and characterisation of various organic polymers under the guidance of senior investigators in the team. The position is funded by the Science and Engineering Research Council for a period of two years. The salary will be at an appropriate point (age related) on the University 1A scale. Applications, including a CV and the names of two referees, be sent to **Mr. J. Deakin, Secretary of the Department of Physics, Cavendish Laboratory, Madingley Road, Cambridge CB3 0HE,** quoting ref. RHF, not later than 30 September. (1377)A

UNIVERSITY OF EXETER (U.K.) UNIVERSITAET MARBURG (F.R.G.) INSTITUT JACQUES MONOD (PARIS) BIOCHEMISTS/MOLECULAR BIOLOGISTS/IMMUNOLOGISTS

Applications are sought for a number of vacancies at postgraduate or post-doctoral level funded by a major collaborative grant under the European Community's SCIENCE Programme for a period of 3 years to investigate the control of gene expression by steroid hormones. The project concerns the characterization of insect steroid (ecdysteroid) receptors, the receptor genes and hormone-responsive genes in the fruitfly *Drosophila melanogaster* and the blowfly *Calliphora vicina*. The collaborating groups are those of Prof. J. Koolman (Marburg), Dr. J-A. Lepesant (Paris) and Dr. L. Dinan (Exeter). The vacancies in Exeter and Marburg are suitable for applicants with a background in biochemistry/organic chemistry or immunology and the vacancy in Paris is appropriate for a molecular biologist. Previous research experience of steroid hormones, receptors, monoclonal antibody production, modern protein purification methods or molecular biological techniques would be an advantage. Applicants must be nationals of one of the twelve European Community countries. Salaries will be on the national scale appropriate for the country, with allowance for age and experience. Travel funds are available for research visits and discussion meetings between the collaborating laboratories.

Applications (3 copies), including details of previous experience, a list of all publications, copies of publications (if available), the names of 3 referees and stating which post(s) you wish to be considered for, should be sent as soon as possible to **Dr. L. Dinan, Dept. Biological Sciences, University of Exeter, Perry Road, Exeter, Devon, EX4 4QG, U.K.,** from whom further particulars may also be obtained (Tel: [0392] 264605 or 264603, Fax: [0392] 263108, Telex: 42894 EXUNIV G). (1399)A

MAGNETIC RESONANCE SPECTROSCOPIST

Position open for PhD magnetic resonance spectroscopist with a primary interest in structure/function of membrane proteins. Applicant must have a strong background and experience in both the theory and instrumental aspects of NMR and ESR and experience in the biochemistry of membrane systems. Work will involve spin label investigations of membrane protein structure. The position will carry the academic title of Assistant Research Ophthalmologist. The successful applicant will be expected to develop a research program in the above area and obtain independent funding to support the research. Starting salary is $37,000/yr. Send CV and references to **Dr. Wayne L. Hubbell, Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024.**

The University of California is an Equal Opportunity/Affirmative Action Employer. (NW4094)A

UNIVERSITY OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY RESEARCH ASSOCIATE RESEARCH TECHNICIAN (Grade 3)

Two research posts are available in the Department of Molecular Biology to join a group working on the molecular biology of *Drosophila* development. The research associate post is available for 7 months in the first instance, salary scale £9,816-£12,381; the technical post for 6-9 months, salary scale £7,479-£8,645.

Please send c.v. including names and addresses of two referees to **Dr. M. Bownes, Department of Molecular Biology, Mayfield Road, Edinburgh EH9 3JR.** *Please quote reference no. 5699.* (1395)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUY'S AND ST THOMAS'S HOSPITALS

Research Assistant — Pharmacokinetics

required to assess the pharmacokinetics of various radio-labelled drugs, to develop and refine the techniques used and to investigate the factors that influence drug levels in these systems.

Applicants must have a BSc or equivalent in pharmacology or allied science. The appointment commences in October in the Division of Pharmacology on the Guy's Campus and is a three year appointment offering the opportunity for a suitable candidate to register for a PhD.

Salary up to £11,466 inclusive per annum. For further information please contact Dr B V Robinson on 01-955 4239.

Application by full CV to the **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting ref G/Pharm/397 by 14th September 1989. (1374)A

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF OXFORD

GLYCOBIOLOGY UNIT

Bio-Organic Mass Spectrometrist BC/304

The Glycobiology Unit is seeking a bio-organic mass spectrometrist with experience in the structural characterisation of biomolecules, especially complex carbohydrates, to supervise the mass spectrometry facility within the Unit. The successful candidate must have extensive experience with sector instruments, as he/she will be responsible for the selection and purchase of a state-of-the-art, high-mass instrument with array detection, and for the operation and routine maintenance of the instrument when it is installed. Experience in GC-MS analysis of carbohydrates using quadrupole instruments would also be advantageous. Collaboration with other members of the Unit in the structural characterisation of complex carbohydrates, and development of new methods for the application of mass spectrometry to the characterisation of biomolecules will be expected. At least two years of relevant post-doctoral experience is essential, as the appointment will be made at a level at least commensurate with that of a junior lecturer. There will be the option of participating in the teaching programme within the Department of Biochemistry. Salary will be on the scale £10,458-16,665 p.a. depending upon age and experience. The appointment will be available for five years in the first instance with the possibility of renewal. Applications which should include a full c.v. and the names of two or three references, should be sent to the Administrator.

An equal opportunity employer. (1320)A

DEPT OF BIOCHEMISTRY, SOUTH PARKS ROAD,OXFORD,OX1 3QU.

SCIENTIFIC OFFICER

The Arthritis and Rheumatism Council for Research requires a Scientific Officer to implement the Council's scientific and research policy and to monitor, through appropriate Chairmen and the General Secretary, the expanding programme of research into rheumatic disease currently running at £7.5 millions per year.

The post is on a five years' contract initially and is open to either non-clinical scientists or clinicians with wide experience in medical research. The hours of work will be at least three days per week, but the eventual hours will be negotiable with the successful candidate.

The salary will be based on appropriate academic scales and a Company car will be provided. The appointee may be based outside London, but visits to London to attend quarterly meetings and to see Chairmen are essential.

Job description and other conditions of service are available from:

The General Secretary, (Scientific Officer),
The Arthritis and Rheumatism Council for Research,
41 Eagle Street, London, WC1R 4AR

to whom applications with detailed Curriculum Vitae and the names of three referees must be submitted by Friday, 13th October, 1989. (1366)A

CHAIR, DEPARTMENT OF MICROBIOLOGY & IMMUNOLOGY

THE SCHOOL OF MEDICINE OF THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL seeks an individual committed to academic excellence to provide professional and administrative leadership for the research and teaching programs of the Department of Microbiology & Immunology. The department has an excellent faculty, strong research and graduate educational programs, and productive collaborative relationships with other departments, research centers and institutes within the University. Other research-intensive institutions in the Research Triangle Park area enhance the scientific environment.

Preference will be given to candidates who reply before October 10, 1989. Reply with curriculum vitae and a list of at least three references to **R. L. Juliano, Ph.D., Chair, Search Committee for Chair of Department of Microbiology & Immunology, Office of the Dean, The School of Medicine, CB# 7000, MacNider Bldg., The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7000.** Women and minorities are encouraged to apply and to identify themselves. UNC-CH is an Equal Opportunity/Affirmative Action Employer. (NW4083)A

Nestlé

Are you looking for a new dimension in your professional activities?

Our Food Products Development Center LINOR, situated in Orbe, Switzerland, about 20 miles north of Lausanne, can offer you such an opportunity.

You already have:

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— an experience of applying chemical engineering principles to solving practical problems in the food industry
— the ability to communicate with people from different background

Then you are the

PROJECT LEADER

COFFEE

we are looking for.

Your main activities will be:

— process and product development for NESTLE companies throughout the world
— critical analysis of unit operations at a pilot plant level
— technical assistance to factories.

If you have a university degree in chemical engineering and a basic knowledge of French, please write with career details to

NESTEC LTD
LINOR Food Products Development Center
CH-1350 ORBE (W6428)A

University of Sussex

SCHOOL OF BIOLOGICAL SCIENCES

POSITIONS IN MOLECULAR BIOLOGY

1. ***AFRC-funded Plant Molecular Biology Programme***
   We are seeking a postdoctoral research assistant, RA1A (£10,458–£16,665) and a grade D technician (£8,645–£10,632) to work on a collaborative three year project with Drs. A.L. Moore, F.Z. Watts and J.F. Burke. The project entails the cloning of *Arabidopsis* genes by complementation of yeast mutations and the subsequent DNA sequence analysis of these genes.
2. ***MRC-funded Invertebrate Molecular Neurobiology***
   This project involves the cloning and DNA sequence analysis of invertebrate genes encoding Alzheimer's disease-related proteins. Applications are invited for a postdoctoral research assistant RA1A (£10,458 –£16,665) and a grade D technician (£8,645–£10,632) to work with Dr. F.Z. Watts. Both MRC positions are for one year, in the first instance.

Experience in molecular biology would be preferred by is not essential. For further details about either project contact Dr. F.Z. Watts (0273 606755 ext. 2676). Applications for these posts, which are available immediately, should include a c.v. and the names and addresses of two referees, and be sent to **Dr. F.Z. Watts, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG.**

AN EQUAL OPPORTUNITY EMPLOYER. (1381)A

UNIVERSITY OF BRISTOL

POSTDOCTORAL RESEARCH

A vacancy exists immediately for a 2 year postdoc. within the School of Chemistry. The applicant should be interested in interdisciplinary research. The objective would be to study pathways of chlorophyll degradation. The applicant should have an interest in HPLC methods. Optionally chemometrics (computational methods in chemistry) and preparative organic chemistry will be assets, although collaborative support is available if needed including opportunities for travel to other European countries. Salary on SERC RA1A scale. Please send curriculum vitae and name of 2 referees to **Personnel Office of Bristol BS8 1TH** quoting reference A638. (1379)A

The University of Queensland

Equal Opportunity in Employment is University Policy

Post Doctoral Research Fellow
(Molecular Biology/Virology)

A post-doctoral research fellow with experience in molecular biology or virology is sought to join an established group of eight scientists and students working on human papillomavirus and its association with cervical cancer. The post is based within the Lions Research facilities in the University Department of Medicine at the Princess Alexandra Hospital, a major teaching hospital in metropolitan Brisbane. The research group has just moved to new laboratories, and on-site facilities include peptide and oligonucleotide synthesis, high quality tissue culture and virologic containment facilities, flow cytometry, and all the expected facilities in a major research institution. The applicant will be expected to contribute to a team working on the regulation of expression of papillomavirus proteins and their immunogenicity. An appointment of two years is envisaged in the first instance, but a suitable applicant would be encouraged to apply for a more permanent position, based on NH&MRC funding. The Princess Alexandra Hospital maintains close research links with other major research institutions in Brisbane, including the Queensland Institute of Medical Research, and the basic science Departments in The University of Queensland. Enquiries should initially be directed to Dr Robert Tindle, Senior Scientist, Lions Immunology Laboratories, Princess Alexandra Hospital, Wooloongabba, Queensland, Australia (Tel 61 07 240 2251: Fax 61 7 240 5399) or to the Head of the Unit, Associate Professor Ian Frazer, who is currently on sabbatical c/- Department of Pathology, University of Cambridge, United Kingdom.

Salary according to qualifications and experience in the range $27,138 - $30,882. Some assistance with travel costs may be available. Applications, including curriculum vitae, copies of significant publications, and names of two professional referees should be submitted to Mr Phil Berquier, Department of Medicine, Princess Alexandra Hospital, Brisbane by 30th September 1989. Successful applicant will be expected to commence duties from the 1st February 1990. Reference No: 38089. (W6429)A *IHF:NSA1669*

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee DD2 5DA
HIGHER SCIENTIFIC OFFICER
(Band I)
3 year appointment (IFS)
DIRECTOR'S GROUP

A research scientist is required to undertake a nuclear magnetic resonance spectroscopy investigation of the coat protein of pepper ringspot tobravirus with particular reference to features of the surface of the virus particle and to RNA binding sites.

Tobraviruses are plant viruses that are being studied in Virology and Zoology Departments by several researchers with particular emphasis on the transmission of the virus by vector nematodes. This project is related to these studies and also to a programme of work on the development of biological applications of NMR spectroscopy. It will be conducted under the joint direction of Drs B A Goodman and M A Mayo.

Salary: £10,026-£13,460 (under review)
Non-contributory superannuation.

Qualifications: First or upper second class honours degree in a relevant physical or biological science with appropriate post-graduate experience.

The Institute is an equal opportunities employer.

Further particulars may be obtained from the **Personnel Officer**, to whom a *Curriculum vitae* with the names and addresses of three referees should be sent by 8th Sept. '89 quoting the appropriate reference (Ref. DG/2/89). (1408)A

ASSISTANT/ASSOCIATE PROFESSOR

The Department of Microbiology and Immunology of the University of Michigan Medical School seeks an immunologist for a tenure-track appointment as Assistant/Associate Professor. The successful applicant will establish an independent research program applying modern molecular/cell biology approaches to the study of the immune response. The position requires active participation in teaching of graduate and medical students. Salary support, a well-equipped laboratory and start-up funds are available from departmental resources. Send C.V., a brief statement of research interests and names and addresses of three referees by January 30, 1990 to:

Dr. J. Latham Claflin
Immunology Search Chairman
Department of Microbiology and Immunology
The University of Michigan Medical School
M6643/0620 Medical Science Building II
Ann Arbor, Michigan 48109–0620

The University of Michigan is an equal opportunity Affirmative Action Employer. (NW4087)A

MICROBIOLOGIST/ MOLECULAR BIOLOGIST
POSTDOCTORAL POSITION

EUROLYSINE Research laboratory, joint venture between ORSAN (France) and AJINOMOTO (Japan) is located in Orsay University campus, 20 km south of Paris.

We are now recruiting a MICROBIOLOGIST/MOLECULAR BIOLOGIST to develop DNA recombinant strains for amino acid production.

Position is initially offered for two years. Application should be sent to:

EUROLYSINE

Dr Daniel Pardo, Eurolysine Research Laboratory, Centre Scientifique d'Orsay, Batiment 403, 91405 Orsay Cedex, France.

(W6435)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR GENETICS

POST DOCTORAL RESEARCH ASSISTANT

A post-doctoral position funded by a MRC grant awarded to Stephen Brown and Keith Johnson is available from 1 October 1989. This project will involve comparative mapping studies of the myotonic dystrophy (DM) region of human chromosome 19 and the proximal region of mouse chromosome 7. The aim of the work is to delineate by genetic and physical mapping methods the region of the mouse chromosome containing the homologue of the human DM locus as a pre-requisite to generating an animal model of this human disorder. Some experience of molecular biological techniques and genetic theory would be of value but is not essential. Salary will be at the appropriate point on the 1A scale.

If applicants wish further details please contact either Dr. Keith Johnson (01) 723-1252 Ext. 5480 or Dr. Stephen Brown Ext. 5484.

Applications in form of a full C.V. with names and addresses of 2 referees should be sent to **Personnel Department** at above address by 8 September 1989. Please quote Ref: PDRA/BIO. (1401)A

THE ROYAL VETERINARY COLLEGE
University of London
Department of Veterinary Basic Sciences
RESEARCH ASSISTANT (GRADE 1A)

A Research Assistant is required for a project funded by the Muscular Dystrophy Group of Great Britain to study development and differentiation of striated muscle cells. Experience in either immunochemistry or developmental/molecular biology will be an advantage. The appointment is for one year renewable for two further years.

Informal enquiries may be made of Dr Dhoot, telephone 01-387 2897 extension 374.

Salary on Academic Research Scale 1A, ranging from £12,108 to £18,315 per annum including London Allowance. Eligibility for Universities' Superannuation Scheme.

Applications with a c.v. and names of two referees should be sent to the **Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU** (tel no: 01-387 2898). (1400)A

UNIVERSITY OF BRISTOL

AFRC-INSTITUTE OF ARABLE CROPS RESEARCH
LONG ASHTON RESEARCH STATION

APPOINTMENTS IN PLANT MOLECULAR BIOLOGY

Applications are invited for four appointments, funded by the AFRC Plant Molecular Biology Programme, to investigate the molecular biology of plant hormone action and the control of cell division. Projects and principle investigators are:

PG507] The identification and cDNA cloning of gibberellin receptors, Dr Richard Hooley.

PG525] A molecular approach to the study of gibberellin stimulated internode extension growth in rice, Dr Andy Phillips.

PG526] The mechanism of ABA-modulated gene expression in oat aleurone cells, Dr Alison Huttly.

PG522] Genes controlling cell division in plants, Dr M Kreis and Prof. P.R. Shewry.

Applicants should have, or expect to gain, a Ph.D in a relevant subject. Appointments will be for a period of three years at the Higher Scientific Officer grade, salary £10,026-£13,460 (pay award pending) non-contributory pension scheme.

Further particulars and application forms from the Head of Administration, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF., to be returned no later than 7th September, 1989.

Informal telephone enquiries can be made to the principal investigators at Long Ashton Research Station (Tel: 0272 392181).

(1386)A

NATURAL ENVIRONMENT RESEARCH COUNCIL

ISOTOPE GEOLOGY CENTRE

Salary upto £18k+

Applications are invited for three positions in ISOTOPE GEOCHEMISTRY commencing on or after 1 November 1989. The Natural Environment Research Council (NERC) undertakes research in the various disciplines of the environmental sciences. The Isotope Geology Centre supports this research principally in the earth sciences, but is now extending its activities into the environmental, marine, and life sciences.

The Isotope Geology Centre will move from its present facilities in London to new purpose-built laboratory facilities at Keyworth (Nottinghamshire) at the end of 1989. NIGC presently has the capability of applying a wide range of both radiogenic (Rb-Sr, Sm-Nd, U-Pb, and conventional K-Ar) and stable (H,C,N,O, & S) isotope methodologies.

Vacancies exist for scientists who will provide collaborative scientific support in radiogenic and stable isotope techniques to UK University and NERC Institute Scientists, research student training, and service support to institute overseas programmes. Responsibilities will include isotopic analysis, in-house facility and mass spectrometer maintenance, participation in project planning and reporting, student training, occasional fieldwork, and a small but important element of personal research.

The successful candidates should have a PhD or equivalent qualifications and experience in some area of isotope geochemistry, together with a proven research ability. Experience in one or more of the following areas would be an advantage: (i) U-PB mineral analysis (ii) O-isotope analysis of silicates and oxides, and (iii) isotope ratio mass spectrometry. Individuals with a broad orientation in the geosciences or a particular interest in the instrumental analysis of the field, laser microsampling and ionization technologies, and the development and application of innovative analytical techniques are especially invited to apply.

Salary will be in the range £11,192–£18,139 depending on qualifications and experience. Terms include a non-contributory pension scheme, 37 hour working week and a generous annual leave allowance.

Applications and further information can be obtained from:

Greg Pirt, The Recruitment Section, NERC Scientific Services, No 1 Dock, Barry, S. Glamorgan CF6 6UZ or by telephone to (0446) 737451 quoting reference number MP108. Closing date for applications is 7th September 1989. Interviews will be held in Keyworth on 28–29 September 1989. (1415)A

Natural Environment Research Council

CANCER RESEARCH CAMPAIGN
HUMAN CANCER GENETICS RESEARCH GROUP
UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY

RESEARCH ASSISTANT: MOLECULAR GENETICS

A post is available for a research assistant to join a group working on the fine genetic mapping and cloning, and ultimately the biology, of the gene for the inherited human cancer syndrome, multiple endocrine neoplasia type 2. This post is additional to two advertised and filled earlier this year. The group is housed in new laboratories in the Department of Pathology, in an excellent scientific environment with several groups with complementary interests nearby. The successful applicant will work in a small team under the leadership of a post-doctoral scientist. Applicants with or without a Ph.D. degree will be equally welcome: substantial experience in molecular genetic techniques is, however, essential.

Appointment for 3 or possible 5 years, depending on age and experience, on University Scales £9,196 to £12,381 (or £15,732 for Post-Doc).

Informal enquiries to Dr. Bruce Ponder on 0223 333711.

Applications in writing with cv and names and addresses of two referees to **The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP.** Please quote **11442.** (1418)A

University of Cambridge
Department of Genetics
Postdoctoral Position in the Genetics and Molecular Biology of Development in *Arabidopsis thaliana*

An A.F.R.C. funded postdoctoral position is available in the laboratory of **Dr I.J. Furner, Department of Genetics, University of Cambridge CB2 3EH.** The successful applicant will join an established and active group working on plant molecular biology and development. The position is available from the 1st October for a period of three years. The starting date is negotiable. Applicants should have a Ph.D. (or be submitting a Ph.D. thesis) in plant biology. Experience in plant molecular biology and/or genetics is desirable but not essential. Applications including c.v. and the names of three referees to **Dr Furner** at the above address by 20 September. *The University is an equal opportunities employer.* (1384)A

Department of Genetics, University of Cambridge, Cambridge CB2 3EH

RESEARCH TECHNICIAN

A research technician is required to join an active group working on plant molecular biology, genetics and development. The position will involve tissue culture, molecular biology of *Arabidopsis thaliana*. Experience in this area is desirable but not essential. An interest in plants and good academic background are important. The post is A.F.R.C. supported and will be available for three years.

The salary will be in the range £8866 to £11241 for a suitably qualified person.

Please send c.v. and the names of three referees to **Dr. I. J. Furner** at the above address by 20th September.

The University is an equal opportunities employer. (1382)A

UNIVERSITY OF OXFORD DEPARTMENT OF ASTROPHYSICS

STARLINK SITE MANAGER

Required for the management and operation of a local area VAX cluster computer system at the Oxford node of the SERC Starlink network which is used for the analysis of astronomical data. The successful applicant will be expected to manage the VAX system, provide user support and to develop Starlink software and operating procedures. Candidates should possess a good degree in mathematics or a physical science and have a background in scientific computing. Some knowledge of astronomy and the VMS operating system is desirable.

The appointment will be for three years, with the possibility of renewal, commencing on 1st October 1989 or as soon as possible thereafter. Salary will be on the National IA scale (£10,458-£16,665 p.a.) with USS benefits.

Applications, together with a cv and the names of two referees should be sent by 20th September 1989, to **Mrs. P. Band, Administrator, Department of Astrophysics, Keble Road, Oxford OX1 3RH (Tel (0865) 273302)** from whom further information may be obtained.

Oxford University is an Equal Opportunity Employer. (1422)A

UEA NORWICH

SCHOOL OF BIOLOGICAL SCIENCES AND JOHN INNES INSTITUTE NORWICH

Postdoctoral Research Assistant

A postdoctoral biochemist is required to work on the mechanisms of signal transduction in plant cells. Previous experience in the areas of phosphoinositide metabolism. G-proteins or calcium transport would be desirable but not essential. The post will be on the RA 1A scale and is for 3 years, funded by the AFRC Plant Molecular Biology initiative. It is available from 1st October 1989 or as soon as possible thereafter. The successful candidate will work in association with Dr. A. P. Dawson in the University and Drs. B. K. Drobak and K. Roberts in the John Innes Institute.

Applications (curriculum vitae plus the names and addresses of two academic referees) should be sent to Dr. A. P. Dawson, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ. (1387)A

UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

RESEARCH TECHNICIAN/MOLECULAR MICROBIOLOGY

Applications are invited for the post of research technician (Grade E) to work on the molecular basis and molecular epidemiology of antibiotic resistance in clinical isolates of *Streptococcus pneumoniae*. The post is funded by The Wellcome Trust for 5 years from October 1st on the Technician Grade E scale (£10,632–£12,325). Applicants should preferably have a degree, and several years laboratory experience in molecular genetics, protein biochemistry, or medical microbiology.

Applications, to include a *curriculum vitae* and the names of two referees, should be sent to **Dr. Brian G. Spratt, Microbial Genetics Group, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG (tel. 0273–678309 direct line).**

AN EQUAL OPPORTUNITY EMPLOYER. (1380)A

PAPUA NEW GUINEA INSTITUTE OF MEDICAL RESEARCH

HUMAN GENETICS SECTION

Research Fellow in Molecular Genetics

Applications are invited from holders of a Ph.D. degree (or equivalent experience) to join an active group, led by Dr. Kuldeep Bhatia, investigating the genetics of immune responsiveness with particular emphasis on malaria and other infectious diseases. Strong background in molecular biology is required; some experience in HLA allogenotyping may be an advantage.

The position, funded by the MacArthur Foundation is available from 1 October 1989 for a period of three years. Salaries are in the range of K20,480 and K26,315, depending on qualifications and experience, and are increased annually by C.P.I. indexation. One kina = U.S.$1.14. In addition to salary, a gratuity of 25% of salary earned is payable each year with further deferred gratuity of 5% of salary increasing to 10% in the second year and 15% in third and subsequent years. Taxation of salary is at standard levels but the gratuity is taxed at flat rate of 2%. Excursion or economy fares are paid for the employee and family between place of residence and Papua New Guinea on recruitment and repatriation. In addition, return fares for employee and family are payable every 18 months to place of residence. Free accommodation is provided in Papua New Guinea. Education expenses are paid for 2 children.

Primary school fees in Papua New Guinea are paid. For children attending high school overseas, education allowances are paid. Settling-in and settling-out allowances are paid at the beginning and end of the contract period.

Applications with a full C.V. and names of three referees should be sent to Dr. Michael Alpers, Director, Papua New Guinea Institute of Medical Research, P.O. Box 60, Goroka, E.H.P., PAPUA NEW GUINEA by 15 September 1989. For further details contact Dr. Kuldeep Bhatia (Telephone 712266; Telefax 721998). (W6408)A

CELL OR DEVELOPMENTAL BIOLOGY

The Department of Anatomy and Cell Biology at the *University of Michigan* Medical School invites applications for *one or two tenure track positions* at the Assistant or possibly at the Associate or Full Professor level. We seek individuals with a vigorous research program in which significant problems are approached with contemporary techniques. Teaching can be in neuroscience, histology or gross anatomy. Candidates should send their curriculum vitae, reprints, statement of research interests and have three letters of reference sent to:

Dr. Bruce M. Carlson, Chairman
Department of Anatomy and Cell Biology
University of Michigan Medical School
Ann Arbor, Michigan 48109-0616

The deadline for receipt of applications is October 1, 1989.

The University of Michigan is an equal opportunity employer. (NW4084)A

UNIVERSITY OF SOUTHAMPTON

Child Health

Neonatal Respiratory Research Group

POSTDOCTORAL RESEARCH ASSISTANT

A position is available now for three years to study aspects of lung maturation and development in relation to the breathing problems of preterm infants. This project will involve analyses of lung surfactant phosphatidylcholine synthesis, secretion and turnover in the perinatal period. Experience of HPLC or immunochemical methods would be an advantage.

Salary will be on the 1A scale, £10,458 to £12,381 per annum. Applications containing curriculum vitae and the names and addresses of two referees should be sent to **Dr. Tony Postle, Child Health, Level C Centre Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY.** Further details may be obtained by telephone (0703) 777222 Ext. 4336. (1369)A

UNIVERSITY OF EDINBURGH

Departments of Chemistry and Microbiology

A POSTDOCTORAL RESEARCH FELLOWSHIP TO WORK ON THE ENZYMOLOGY OF FLAVOCYTOCHROME b_2

Applications are invited for a postdoctoral position to work on the enzymology of electron transfer in flavocytochrome b_2, a lactate dehydrogenase from yeast. The project will involve rational modification of the enzyme by site-directed mutagenesis and by constructing hybrid enzymes using fragments of the flavocytochrome b_2 gene from two different species of yeast. The altered enzyme will be characterized by biochemical and spectroscopic methods in order to probe the mechanisms of the various electron-transfer steps within the enzyme.

Applicants should preferably have some experience in biochemistry, molecular biology, or bioinorganic chemistry. Salary will be on the RA1A scale at a point depending on qualifications and age. The appointment will be for a period of up to 3 years. Applications including a C.V. and the names and addresses of two referees should be made to: **Dr S.K. Chapman, Dept of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ;** or **Dr. G.A. Reid, Dept of Microbiology, University of Edinburgh, West Mains Road, Edinburgh EH9 3JG.** Informal enquiries can be made to Dr S.K. Chapman tel. 031-667-1081 ext 3671. PLEASE QUOTE REFERENCE NO. 5700 (1417)E

The Wolfson Centre has a vacancy for an

ELECTROCHEMICAL ENGINEER

to join their applied electrochemistry group based at Southampton University.

Candidates should possess a PhD in electrochemical engineering and preferably have post degree engineering experience.

Salary: up to £16,665 per annum, depending on age, experience and qualifications.

Written application including a CV (2 copies) and the names and addresses of 2 referees should be forwarded to: **Mr H F Watson, Staffing Department, The University, Highfield, Southampton SO9 5NH** quoting reference number **10/HFW/SMT.** Closing date: 8 September 1989. (1368)A

AFRC Institute of Animal Physiology and Genetics Research

Edinburgh Research Station

Postdoctoral Research in Molecular Genetics

The research station has a substantial programme in molecular genetics including a major research effort in the transgenic biology of laboratory and farm animals (see Nature 328, 530–532; 331, 70–72; Biotechnology 6, 179–183; 7, 48 7–492). We have embarked on a major expansion in this area and are applying techniques of molecular biology and gene transfer to critical problems of gene regulation, physiology and development. Within this programme the following post-doctoral positions have been created.

1. Germline transformation of poultry: A molecular biologist is required to join a project to develop a method to produce transgenic poultry by injection of DNA at the single cell stage, followed by in vitro embryo culture (see Nature 331, 70–72). The project will involve developing methods to detect integration of injected sequences and also an investigation of the possible application of sperm mediated-DNA transfer to poultry. For further information contact Dr. Helen Sang on 031–440–2726.

2. Molecular biology of avian prolactin: A molecular biologist is required to join a programme that involves both molecular biologists and physiologists, investigating the control and function of avian prolactin. The project will involve analysis of the control of prolactin gene expression by VIP, and the function of prolactin in broodiness in hens. For further information contact Dr. Helen Sang or Dr. Peter Sharp on 031–440–2726.

3. Alteration of milk composition in transgenic animals: The project will involve targeting expression to the mammary gland of casein genes and their genetically engineered derivatives, and assessing the effects of milk composition and properties. The work will be carried out in collaboration with the Hannah Research Institute, Ayr. The person appointed will have experience in recombinant DNA techniques. For further information contact John Clark on 031–667–6901.

The Research Station occupies new laboratories just outside Edinburgh and carries out basic research in animal genetics, molecular biology and physiology; it has close links with the biology departments of the University of Edinburgh including the newly formed IRC for Animal Genome Research.

Qualifications: First or Upper Second Class Honours degree in a related discipline plus 2 years post graudate experience for entry at Higher Scientific Officer level. 5 years post graduate experience is required for entry at Senior Scientific Officer level.

Condition: All posts are tenable for three years in the first instance. Salary is HSO scale £10,678–£14,335, 22 days annual leave; in SSO scale £13,254–£18,139, 25 days annual leave.

Application forms (specify which posts) can be obtained from **Mrs. L. Hunter, Personnel Officer, AFRC Institute of Animal Physiology and Genetics Research, Edinburgh Research Station, Roslin, Midlothian EH25 9PS,** and should be completed by 8th September 1989. (1416)A

UNITED MEDICAL AND DENTAL SCHOOLS

of Guy's and St Thomas's Hospitals

Guy's Campus — close London Bridge

Research Technician

Applications are invited for the post of Research Laboratory Scientific Officer in the Division of Medicine (Rheumatology Unit). The post is supported by a grant from the Arthritis and Rheumatism Council to investigate the molecular basis of leukocyte-endothelial cell interactions. Initial funding is for two years. Experience in tissue culture and monoclonal antibody technology an advantage but not essential. Salary in the range £7,605-£9,941 pa inc depending on qualifications and experience.

For informal inquiry please ring Dr D Haskard on 01 955 5000 ext 3678/3691. To apply, please send two copies of your CV, together with the names and addresses of two referees to: **The Personnel Officer, UMDs, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH,** quoting ref no G/MED/392.

Closing date 7/9/89. (1372)A

UNIVERSITY OF NATAL

DURBAN
SOUTH ARICA

The University of Natal rejects apartheid. It is an equal opportunity, affirmative action University.

Department of Chemistry and Applied Chemistry

Professor of Organic or Inorganic Chemistry

Ref D89/89
Closing date: 10 November 1989

The remuneration package includes a negotiable salary, 13th cheque, subsidised housing scheme if eligible, remission of University fees, generous relocation costs and substantial retirement benefits, including a tax-free gratuity and a favourable annuity under the pension scheme. Applicants will be supplied with further details.

The Department of Chemistry and Applied Chemistry in Durban consists of Divisions of Applied (including Analytical), Inorganic, Organic and Physical Chemistry. It is an autonomous unit with a non-permanent Head and this post is at present under review. Applicants for the present post should state their interest in the Headship.

The commencing salary notch will be dependent on the qualifications and/or experience of the successful applicant.

Application forms, further particulars of the post and conditions of service, including details of fringe benefits are obtainable from the Secretary, South African Universities Office, 2nd Floor, 16 Charles II Street, London SWIY 4QU or the Personnel Section, University of Natal, King George V Avenue, Durban 4001, South Africa, with whom applications on the prescribed form must be lodged, quoting the relevant reference number.

(W6430)A

University College London
Department of Biochemistry
Medical Molecular Biology Unit

POSTDOCTORAL RESEARCH ASSISTANT (Scale 1A)

We have a vacancy for a postdoctoral scientist to join a group applying a molecular and cell biological approach to the study of positional signalling and pattern formation in the chick limb and face.

The successful candidate will concentrate on examining the structure, expression and function of a novel chick insulin-like growth factor II gene product.

The post is funded by the Medical Research Council for three years and is available from 1 October 1989. It will be held in a well-equipped laboratory housing some 30 research workers applying recombinant DNA techniques to medical and biological problems.

Applications, with curriculum vitae and the name of two referees, should be sent before 14 September 1989 to **Dr Paul Brickell, Medical Molecular Biology Unit, Windeyer Building, Cleveland Street, London W1P 6DB.**

Equal Opportunities Employer (1406)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

POSTGRADUATE/POSTDOCTORAL RESEARCH SCIENTIST IN BIOANALYTIC CHEMISTRY

Applications are invited for the above Arthritis and Rheumatism Council funded post which is available for three years. The project involves the development and assessment of novel fibre-optic probes for the in vivo measurement of reactive oxygen radical species in biological fluids of patients with inflammatory joint disorders, and forms part of a collaborative programme involving Drs MK Shepherd and DE Spillane at the Polytechnic of North London. Applicants should possess a minimum of good honours degree in chemistry, biochemistry or a related subject, and should preferably have experience of modern analytical/bioanalytical chemistry.

The salary will be £11,466-£14,036 for a postgraduate and £12,018-£18,315 for a post-doctoral appointment.

Further particulars can be obtained from Dr Martin Grootveld or Professor David Blake (01-377 7765). CV and names and addresses of two referees should be sent to **Miss S Bailey, Office Manager, ARC Building, London Hospital Medical College, 25-29 Ashfield Street, London E1** within 14 days. (1373)A

STAFF POSITIONS

DEPARTMENT OF CELL AND DEVELOPMENTAL BIOLOGY

ROCHE INSTITUTE OF MOLECULAR BIOLOGY

As part of a newly expanded program in developmental biology, applications are invited for two positions equivalent in rank to Assistant and Associate Professor. Candidates are sought who are applying contemporary experimental approaches to basic research in development biology. Institute staff enjoy total freedom and independence in their choice and pursuit of research problems, as well as full funding of their research programs, including postdoctoral fellows, by Hoffmann-La Roche. The institute is located in a suburban setting only 10 miles west of New York City. Send curriculum vitae, names of three references, and summary of research interests to: **Dr. Paul Wasserman, Chairman, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, New Jersey 07110.** We are an equal opportunity employer. (NW4098)A

ROCHE **Roche Institute of Molecular Biology**
Hoffman-La Roche Inc.

CHAIRPERSON

Department of Meteorology

University of Maryland, College Park

Nominations and applications are invited for the position of Chairperson of the Department of Meteorology at the University of Maryland at College Park. Since the previous search, the state has mandated the enhancement of the College Park campus to make it into the flagship campus of the University System. The chairperson will be expected to contribute to this enhancement by attracting high-quality faculty for additional tenure-track and tenure positions, by providing imaginative leadership, and by promoting the department in the emerging national and international atmospheric and oceanographic science programs. The candidate should possess a doctoral degree and a record of excellence in scholarly achievements.

Applications should be accompanied by a curriculum vita and the names of four references. Send to: **Dr. Donat G. Wentzel, Meteorology Chair Search Committee, Astronomy Program, University of Maryland, College Park, MD 20742.** To be assured of full consideration, applications should be received by October 15, 1989. *EOE/AAE.* Women and minority candidates are encouraged to apply. (NW4088)A

FACULTY POSITION IN MOLECULAR GENETICS

The Wilmer Institute of The Johns Hopkins University, School of Medicine, invites applications for a full time faculty position at the level of Assistant Professor, Associate Professor, or Professor. We are particularly interested in established investigators in the field of molecular genetics of eye disease in human patients and in animal models. Scientists with a strong background in other aspects of molecular genetics or molecular biology, and who are willing to move into eye research, are also encouraged to apply. A detailed curriculum vitae, including a list of publications and current grant support, a description of current research interests, and three (3) letters of recommendations should be addressed to:

Ruben Adler, M.D., Chairman, Search Committee
The Johns Hopkins University School of Medicine
The Wilmer Institute
Maumanee 519, 600 North Wolfe Street
Baltimore, Maryland 21205

The Johns Hopkins University is an affirmative action/equal opportunity employer (NW4102)A

UNIVERSITY OF BRISTOL

Department of Geology

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a 3-year postdoctoral position to assist Dr D. E. G. Briggs in a NERC-funded project on the taphonomy of exceptionally preserved fossil biotas. The emphasis will be on an experimental investigation of decay inhibition and early diagenetic mineralisation in association with soft-bodied invertebrates.

Candidates should preferably have experience in sedimentary geochemistry, sedimentry microbiology, or palaeontology/taphonomy, but individuals with other relevant backgrounds are encouraged to apply.

Salary within Research 1A scale, £10458-£12381.

For further details telephone Bristol 303136 (ansaphone after 5.00 p.m.) or write to the **Personnel Office, Senate House, University of Bristol, Bristol BS8 1TH.** Please quote reference A657. (1412)A

ASSISTANT PROFESSOR OF PLANT PATHOLOGY

The Botany Department at the University of Toronto invites applications for a tenure-track position in plant pathology at the assistant professor level, starting July 1, 1990. The successful candidate should have a Ph.D. and, preferably, post-doctoral experience. He/she will be expected to develop an active research programme in the area of host-parasite interactions at the molecular, cellular, genetical, biochemical or physiological levels. Teaching responsibilities will include all or part of an introductory undergraduate plant pathology course and participation in team-taught undergraduate courses in appropriate areas of biology. Applicants should submit a curriculum vitae and the names of three referees by October 31, 1989 to: **Dr. J.A. Hellebust, Chairman, Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2, FAX – (416) 979-5878.** In accordance with Canadian Immigration regulations, this advertisement is directed towards Canadian Citizens and Landed Immigrants. The University of Toronto encourages both women and men to apply for this position. (NW4106)A

University of Cambridge

Department of Pharmacology

2 Post-doctoral Research Associates

Applications are invited for work on the following projects:

(1) BDA grant to Dr M L J Ashford on the role of potassium channels in the control of vetromedial hypothalamic neurone firing in response to changes in extracellular glucose concentrations. The appointment will be for two years initially at a starting salary of up to £13,527.

(2) MRC grant to Dr M L J Ashford and Professor S K Smith (Department of Obstetrics and Gynaecology) on pharmacological modulation of potassium channel activity and the control of human myometrial excitability. The appointment will be for three years with a starting salary of up to £12,381.

Patch clamp experience is desirable for both positions. Applications should reach Dr M L J Ashford by 29 September at the above Department, **Tennis Court Road, Cambridge CB2 1QJ.** Informal enquiries may be made by telephoning 0223 334000 or FAX 0223 334040. (1409)A

POSTDOCTORAL POSITIONS PROTEIN CRYSTALLOGRAPHY

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

The Center for Macromolecular Crystallography has two postdoctoral positions available for a collaborative project with Schering Corporation on crystallographic studies of protein lymphokines (including interferons, interleukins and colony stimulating factors), their receptors, and the protein/receptor complexes. Crystals of two lymphokines (y-interferon and GM-CSF) suitable for structural analysis are now in hand.

Fellowships are in the range of $26,000-$40,000, depending upon the experience and qualifications of the individuals. Initial appointments will be for a one-year period, with possible extension for additional years.

Send application letter with current CV and two references to: **Steven E. Ealick** or **Charles E. Bugg, Center for Macromolecular Crystallography, The University of Alabama at Birmingham, Box 79 THT, Birmingham, AL 35294.** (NW4100)A

University of Cambridge
Department of Biochemistry
SERC CENTRE FOR MOLECULAR RECOGNITION: DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSOCIATE: PROTEIN-DNA INTERACTIONS

Applications are invited for two postdoctoral positions. One position is available from October 1st 1989 for up to three years and will be for work primarily on various aspects of histone-DNA interactions in chromatin structure, superstructure and function. The other, for work on the role of the non-histone proteins HMG 1 and 2, will be available from December 1st 1989 for up to 17 months. Both starting dates are negotiable. Salary in the range £10,458-£15,372 p.a. according to age and experience. Candidates should have an interest in macromolecular structure and interactions and a strong background in molecular biology, biochemistry or related subject. Experience in expression of cloned genes, or in NMR spectroscopy may be advantageous.

Applications enclosing a C.V. and the names and addresses of two referees should be sent as soon as possible to **Dr. J.O. Thomas, Department of Biochemistry, Tennis Court Road, Cambridge, CB2 1QW** to whom informal enquiries may be made (Tel: (0223) 333670).

THE UNIVERSITY FOLLOWS AN EQUAL OPPORTUNITIES POLICY (1376)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG
DEPARTMENT OF BIOCHEMISTRY & MOLECULAR GENETICS

GRADUATE RESEARCH ASSISTANT

A research assistant position is available for 3 years from October 1989 on an MRC funded grant to study the shaker-1 mutant in mouse, a locus involved in hearing impairment. The project will run in collaboration with Dr. Karen Steel at the MRC Institute for Hearing Research, Nottingham. The work involves the detailed long-range genetic and physical mapping of the shaker-1 locus on mouse chromosome 7 involving mouse genetic crosses (see Genomics 1, 153-158 [1987], pulse-field gel elecrophoresis and YAC cloning. Applicants should have a degree in an appropriate background. Salary is on the 1B scale (starting point: £9816 pa + £1650 pa London Allowance).

Enquiries may be made to Dr. S. D. M. Brown on (01) 723-1252 Ext. 5484.

Applications including a C.V. with names and addresses of two referees should be sent to **Personnel Department** at above address. Please quote Ref: GRA/SDM. (1403)A

SENIOR NEUROBIOLOGIST/NEUROSCIENTIST

National Institute of Mental Health Intramural Research Program

The NIMH is accepting applications for a senior neurobiologist/neuroscientist with interests in studying basic brain mechanisms of behavior. Applicants must hold a doctoral degree or equivalent and be interested in joining an established research program involving behavioral neurophysiology, neuroanatomy, neuropsychology, developmental neurobiology, neuroethology and other disciplines. The laboratory facilities are located in a rural setting approximately 30 miles west of the main NIH Bethesda campus near the town of Poolesville, Maryland. Researchers in the fields of neurophysiology, neuroanatomy, experimental psychology (including physiological psychology), developmental neurobiology, and related fields in the brain and behavioral sciences are especially encouraged to apply. Excellent facilities exist for primate research. The successful candidate should have broad experience and established credentials in the neurosciences. Duties will include scientific leadership and administration of a laboratory, as well as conduct of an internationally-recognized research program. Salary will be commensurate with other NIMH researchers having similar responsibilities, approximately $57,158 to $75,500 plus full Federal benefits. Send C.V., bibliography, a statement of future research directions, and the names of three references to:

Steven M. Paul, M.D.
Acting Director, Intramural Research Program
National Institute of Mental Health
Building 10, Room 4N-224
9000 Rockville Pike
Bethesda, Maryland 20892

NATIONAL INSTITUTES OF HEALTH

NIMH IS AN EQUAL OPPORTUNITY EMPLOYER (NW4089)A

U.S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE
SOUTHERN FOREST EXPERIMENT STATION

PROJECT LEADER/SUPERVISORY RESEARCH ENTOMOLOGIST, RESEARCH FORESTER, RESEARCH ECOLOGIST, RESEARCH BIOLOGIST.

USDA-FS, South. For. Exp. Sta., is seeking a Research Entomologist, Research Forester, Research Ecologist, or Research Biologist to lead a team of researchers studying the population dynamics and interactions of the southern pine beetle, host trees, and stands to improve management of the pest and to develop practical means of control. Salary range is GM-13-15 ($41,121 to 57,158). Information concerning the position may be obtained from **Personnel Management, Rm. T-10210, USPSB, 701 Loyola Avenue, New Orleans, LA 70113 (504/589-3921).** The USDA is an Equal Opportunity Employer. (NW4092)A

UNIVERSITY OF GLASGOW
DEPARTMENT OF PATHOLOGY
WESTERN INFIRMARY

POSTDOCTORAL RESEARCH ASSISTANT

Post Doctoral Research Assistant required to work on the biosynthesis of complement components by cells in the synovial membrane.

Experience with molecular biological techniques is preferable and experience with tissue culture would be advantageous. This is a three year grant funded post starting at £11,088 p.a. on the University 1A scale. Please send applications with C.V. and the names and addresses of two referees to **Professor Keith Whaley, Pathology Department, Western Infirmary, Glasgow G11 6NT.** (1413)A

POSTDOCTORAL/RESEARCH ASSOCIATE

available for the study of the mechanism of interaction of antibiotics with proteins. Preference will be given to individuals with a strong background in the isolation and structural identification of drug conjugates. Salary commensurate with experience. Send curriculum and names of three references to: **Dr. Harold L. Kohn, Department of Chemistry, University of Houston, TX 77204-5641.** *An Equal Opportunity Employer.* (NW4095)A

The Imperial Cancer Research Fund is the largest independent cancer research institute in Britain with wide ranging intersts in fundamental, applied and clinical research. Vacancies now exist at various levels in a range of departments for experienced staff:

HEAD OF AMINO ACID SEQUENCING SERVICE

£17,800-£21,100

We need someone with considerable technical and scientific experience to run the Fund's Amino Acid Sequencing Service. The service is based upon up-to-date Applied Biosystems instrumentation. You will be expected to work independently to isolate peptides and/or develop suitable transfer systems, to obtain and interpret reliable sequence data and to interact with scientists in the research laboratories. You will also be responsible for ensuring that the laboratory is kept up-to-date with new techniques and instrumentation. Experience in the degradation of proteins and purification of peptides on a micro scale and/or the procedures of microsequencing is essential.

Salary will depend on qualifications and experience, but a higher salary would be awarded to an exceptional candidate.

Application should be made by sending a full cv plus the names and addresses of three referees to the address below. Please quote ref 227/R.

TRANSCRIPTION LABORATORY

£11,284-£16,201

The laboratory combines molecular biological and biochemical approaches to the study of gene regulation by growth factors. A Scientific Officer is required to assist on scientific projects studying gene control elements and their cognate DNA binding proteins. Previous experience of molecular biology of nucleic acids, tissue culture, and protein purification essential.

HNC/Degree in a relevant discipline required. Ref: 228/R.

For further information and application form, please write or telephone the **Recruitment Office, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX. Telephone 01-242 0200 ext 3357.** Please quote the appropriate reference.

Smoking is actively discouraged. (1426)A

IMPERIAL CANCER RESEARCH FUND

Transgenic Mouse Colony:

A faculty position is available immediately for a qualified investigator to lead a transgenic mouse research and development program. The primary intent is to develop *in vivo* models for studying the microvascular complications of diabetes. Candidates must have experience in all aspects of transgene manipulations and a strong background in molecular biology. Although experience in diabetes-related research is an asset, it is not necessary.

The Whittier Institute for Diabetes and Endocrinology is a non-profit research institute that has a solid foundation for basic research in islet transplantation, in studies on CNS and pituitary function, in reproductive biology, growth factor biochemistry and in angiogenesis. Send curriculum vitae with a brief statement of career goals to:

Andrew Baird
Chairman, Transgenic Core Search Committee
The Whittier Institute
9894 Genesee Avenue
La Jolla, CA 92037

EOE/AA (NW4091)A

POSTDOCTORAL BIOCHEMISTS/CHEMISTS

Two postdoctoral positions are available in an interdisciplinary team, led by Prof. B.E. Smith, studying the nature, function and binding of the metal clusters in nitrogenases.

Postion 1 is funded for two years by the European Community for a 'Twinning' between the Nitrogen Fixation Laboratory and the Department of Biochemistry, Agricultural University of Wageningen. The project involves a study of the electron paramagnetic resonance (EPR) spectroscopy of oxidized forms of the MoFe protein. Experience in EPR spectroscopy would be an advantage. EC rules stipulate that this position can only be held by non-UK nationals of Member States.

Position 2 is funded by AFRC for two years in the first instance and involves the characterisation of nitrogenases derived from site-directed mutants. Experience in protein isolation and the application of spectroscopic techniques to the study of metallo-proteins or site-directed mutagenesis techniques would be an advantage.

Send CV to Mrs. J.A. Durrant at the AFRC Institute of Plant Science Research, Nitrogen Fixation Laboratory, University of Sussex, Brighton BN1 9RQ.

Closing date: 14/9/89.

The Agricultural and Food Research Council is an Equal Opportunities Employer. (1390)A

AGRICULTURAL & FOOD RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY & GENETICS RESEARCH

BABRAHAM, CAMBRIDGE, CB2 4AT

A Post-doctoral MOLECULAR NEUROBIOLOGIST is required (for a 3 year appointment) to work on gene expression in brain circuits controlling behaviour and/or development. Candidates should have experience in *in situ* hybridization techniques and be enthusiastic about initiating a new area of research in the Department of Behavioural Physiology. The successful applicant will have the opportunity also of collaborative work with molecular biologists and neuroscientists in other Departments at Babraham. Appointment at HSO (£10,678-14,909) or if well qualified SSO (£13,254-18,864). Non-contributory superannuation scheme. 25 days leave and 10½ public holidays a year. Application forms available from the Personnel Office quoting ref: SNT/BRF by 7th September 1989. (1385)A

ROWETT RESEARCH INSTITUTE

Bucksburn, Aberdeen, AB2 9SB

CELL BIOLOGIST/ BIOCHEMIST HSO/SSO GRADE

Applications are invited for t post of cell biologist/biochem within the Skeletal Grow Group. The appointee will join multidisciplinary group with inte ests in the endocrine/paracri control of bone cell activity, cc lagen metabolism and calcium ar phosphorus homeostasis. Canc dates should preferably hav research experience in an area bone metabolism. The perso appointed to this position will hav technical assistance and be e couraged to develop their own lir of research within the remit of th Group.

This is an established positic and the appointment will be fc three years in the first instance a either Higher Scientific Officer c Senior Scientific Officer level wit the grade and salary dependin upon qualifications and expe ience based on the following min mum requirements.

First or upper second class hon ours degree in an appropriate dis cipline with at least 2 years rele vant post graduate experience fo HSO and 4 years for SSO appoint ment.

Salary Range: HSO £10,678 t £14,909; SSO £13,254 to £18,864.

The Institute is an equal oppor tunities employer and a non-con tributory superannuation scheme is in operation.

The Rowett Research Institute is Western Europe's leading cen tre for studies in nutritional sci ence. It is located on the outskirts of Aberdeen and has close links with the other prominent educational and research centres in Grampian Region which is one of Scotland's most thriving and attractive areas.

Applications in writing with a full CV and the names and addresses of 2 referees should be sent to the Personnel Officer at the Institute from whom further details can be obtained. For more informal information, potential applicants are encouraged to contact Dr. Nigel Loveridge (0224) 712751 ext. 174.

Closing date 21st September 1989. (1404)A

THEORETICAL POPULATION ECOLOGIST

The Department of Biological Sciences, University of California, Santa Barbara, invites applications for a tenure-track position at the Assistant Professor level in Theoretical Population Ecology, effective July 1, 1990. Applicants should combine an exceptional and broad background in mathematics appropriate to modelling ecological systems with demonstrated ability to apply these techniques with good biological insight. Ph.D. normally required by time of appointment. The successful candidate will be expected to establish a strong research program, to develop an undergraduate and a graduate course in theoretical population ecology, and to participate where appropriate in other aspects of the department's teaching. The department has a strong and diverse group of population biologists, and evidence that the candidate has a desire and ability to interact and collaborate with other faculty will be an advantage. Candidates with postdoctoral experience will be given preference. Interested individuals should send a copy of their curriculum vitae, a summary of their research goals, and the names of four referees to: **The Chairman, Department of Biological Sciences, University of California, Santa Barbara, CA 93106, USA,** by October 1, 1989. *The University of California is an Equal Opportunity/Affirmative Action Employer. Proof of U.S. citizenship or eligibility for U.S. employment will be required prior to employment (Immigration Reform and Control Act of 1986).* (NW4096)A

HEAD, ACADEMIC UNIT

A new School of Basic Life Sciences is searching for an established researcher with academic experience to direct the Division of Structural and Systems Biology. This unit is involved in a doctoral program with participation in cell biology, neurobiology and molecular biology. Its faculty also teach in the health science professions in the areas of cell biology, anatomy, neurosciences, physiology and histology. A person with an established record of research support in any aspect of modern biochemistry, cell or molecular biology, including molecular immunology and neurobiology, is sought for a tenured position as Division Head and the opportunity to expand a new graduate program revolving around structural and functional aspects of molecular recognition in biological systems.

Applications should be received by November 1, 1989, however, they will be accepted until the position is filled. Send letters of interest, including a curriculum vitae and names of at least three prospective references to:

Dean Marino Martinez-Carrion
School of Basic Life Sciences
University of Missouri-Kansas City
Kansas City, MO 64110

The University of Missouri-Kansas City is an equal opportunity/affirmative action employer. (NW4097)A

JUNIOR/SENIOR FACULTY POSITIONS

MOLECULAR BIOLOGY/BIOCHEMISTRY

The Department of Biochemistry at Indiana University School of Medicine is seeking applicants for two full-time, tenure track positions at the *Assistant, Associate* or *Full Professor* level. Applicants should have a Ph.D., M.D. or equivalent degree and postdoctoral experience in Molecular Biology/Biochemistry. It is expected that the new faculty will develop independent research programs in areas of Molecular Biology/Biochemistry including regulation of eukaryotic gene expression, DNA recombination/gene targeting, DNA replication and repair, DNA-protein interactions, macromolecular structure, cellular and metabolic regulation. Significant space and start-up funds are available. Faculty are involved in both graduate and medical programs. Applicants should submit their curriculum vitae, a description of research plans, and the names of three references to:

Dr. Peter J Roach,
Department of Biochemistry,
Indiana University School of Medicine,
Indianapolis,
Indiana 46202-5122.

Indiana University is an equal opportunity, affirmative action educator, employer and contractor. (NW4104)A

The International Rice Research Institute (IRRI)

seeks a

Farming Systems Agronomist

Duties:

- Identify crop sequences to increase crop diversification and enhance sustainability in irrigated and favorable rainfed rice ecosystems
- Study direct-seeding methods for rice to increase crop intensity and reduce production costs
- Develop procedures to quantify the stability and sustainability of livelihood in irrigated systems
- Collaborate with national programs to strengthen research on irrigated rice systems in the Asian Rice Farming Systems Network
- Participate in both degree and non-degree farming systems training

Qualifications needed:

- Ph.D. in agronomy, soil science, or related discipline
- Two or more years of relevant research experience
- Experience in international agricultural research highly desirable

IRRI is an international, non-profit, agricultural research institute funded by about 30 donor nations and organizations. Its goal is to enhance rice production and sustainability to benefit the world's 1.3 billion people who are dependent on rice for their livelihood and as their basic food.

The person chosen for the position will live at IRRI headquarters on the campus of the University of the Philippines at Los Banos, 60 km south of Manila. All positions require fluency in written and spoken English. International travel is required. Salary and perquisites are internationally competitive with those of similar international institutions and programs in agriculture.

Application due: 31 October 1989. Submit curriculum vitae, date of availability, and the names of three references to **Dr. Klaus Lampe, Director General, IRRI, P.O. Box 933, 1099 Manila, Philippines.** (W6432)A

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

In the Cancer and Polio Research Fund Laboratories. To work on a programme to isolate and characterise a major growth factor for the breast, and to study its role in breast cancer. Experience in purification of proteins is desirable. A dedicated technician is allocated to this project. The project complements ongoing research in this Unit into the growth and differentiation of the normal and neoplastic mammary gland.

The post is tenable for three years in the first instance at an initial salary in the range £10,458 — £12,381 per annum. Informal enquiries to Professor P. S. Rudland, tel. 051-794 4332 or Dr. J. A. Smith, tel. 051-794 4329.

Closing date: 7 September, 1989.

Quote Ref. RV/473/N.

DEPARTMENT OF GENETICS AND MICROBIOLOGY

POSTDOCTORAL SENIOR RESEARCH ASSISTANT

To work on chromatic structure and activity in a group run jointly by Professor J. P. Baldwin at Liverpool Polytechnic and Dr. R.S. Hill at the University. Applicants should have an interest in biological molecular structure and, ideally, a working knowledge of small angle scattering and/or x-ray crystallography. Informal enquiries to Professor Baldwin, School of Information Science and Technology, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF. Tel. 051-207 3581 or Dr. Hill at the University address below. Tel. 051-794 3628/3622.

The post is tenable for up to two years from 1 October, 1989, at an initial salary of either £9,816 or £10,458 per annum.

Closing date: 11 September, 1989.

Quote Ref. RV/491/N.

Applications, by c.v. with the names of three referees, for both the above posts to: The Director of Staffing Services (AS), The University, PO Box 147, Liverpool L69 3BX, from whom further particulars may be obtained.

University of (1419)A

LIVERPOOL

An Equal Opportunity Employer

DIRECTOR OF THE PROTEIN STRUCTURE LABORATORY
UNIVERSITY OF CALIFORNIA, DAVIS

The Department of Biological Chemistry invites applications from qualified individuals for the position of Director of the Protein Structure Laboratory. The Protein Structure Laboratory is a nationally recognized instrumentation facility serving the needs of the entire Davis campus of biologists and biochemists, spanning all Schools and Colleges. The Laboratory houses modern equipment supporting research programs in wide areas of biochemistry biotechnology, and molecular biology. Major instruments of the Laboratory include gas and liquid phase protein sequencers, amino acid analyzers, a peptide synthesizer, an oligonucleotide synthesizer and associated high performance liquid chromatographs. The Director is responsible for the supervision of all Laboratory personnel and for quality control of the services provided by the Laboratory. The Director will have the opportunity to develop and pursue his/her own research program, since the Directorship carries with it an academic appointment within the Department. A modest teaching responsibility is associated with the position. Applicants should possess a Ph.D., or equivalent experience, in biochemistry, chemistry, or an allied field, and have had extensive experience in the operation and management of instruments of the type now present in the Laboratory. Interested applicants should submit a curriculum vitae, a description of research interests and instrumentation and management experience, and provide the names and addresses of 3 references, to be sent to: **William F. Benisek, Chair, Search Committee for Protein Structure Laboratory, Department of Biological Chemistry, School of Medicine, University of California, Davis, CA 95616.** The position is open until filled. Applications will be accepted through 12/31/89, or until a suitable candidate is identified. The University of California is an Equal Opportunity/Affirmative Action Employer. (NW4090)A

GEOFILMS
A GEOLOGIST TO TRAIN AS FILM PRODUCER

Geofilms is the only company, in the UK, specialising in film production for the Oil and Mining Industries. Due to expansion, we need a **geologist to train as a film producer.** The successful applicant would have at least five years post-grad industrial experience and must demonstrate the ability and enthusiasm to communicate all aspects of Earth Sciences. Please apply in writing to:–

John Simmons, Geofilms & Videos Ltd,
Osborn Court, Olney, Bucks, MK46 4AG, UK

This is a rare opportunity for a geological career with a difference (1414)A

ASSISTANT/ASSOCIATE PROFESSOR

Unit for Laboratory Animal Medicine and the Department of Microbiology and Immunology, UNIVERSITY OF MICHIGAN, Ann Arbor, jointly seek a scientist for a tenure-track appointment. The succesful applicant will establish an independent research program applying molecular/cellular approaches to the pathogenesis of infectious diseases. The position entails active participation in teaching graduate and medical students and veterinary postdoctoral fellows. Salary support, a well equipped laboratory and start up funds will be provided.

Send curriculum vitae, a brief statement of research interests and names and addresses of three references by October 15, 1989 to **Bennett J. Cohen, DVM, PhD, Chairman ULAM/Microbiology Search Committee, Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI 48109–0614.**

The University of Michigan is an equal opportunity Affirmative Action employer. (NW4063)A

UNIVERSITY OF WARWICK
MOLECULAR MECHANISMS IN SMELL

The Olfaction Research Group in the Department of Chemistry is pursuing a number of topics on the molecular mechanisms in olfactory cells. There are immediate vacancies for the following positions of interest to biochemists or biological chemists.

Postgraduate Research Assistant

This position would suit either a recent graduate or someone with some research experience. The principal objective of the work is to identify enzymes in olfactory mucosa which might be damaged by the chemicals in polluted air. The mechanisms of the inhibition of the enzymes by xenobiotics will also be studied.

This position is available for two years. It might be possible for a suitable candidate to register for a part-time higher degree.

Starting salary up to £11,088 on the RA1B scale: £9,816–£12,381 pa.

Postdoctoral Research Fellowship

In recent years we have investigated the binding of lectins to olfactory mucosa. This is a possible approach to the identification of olfactory receptors. This project aims to identify the glycoproteins or other components of the sensory membranes which bind the lectins and to examine their status as putative receptor proteins. Experience of working with membrane glycoproteins would be helpful. This project may involve collaboration with other research groups.

This position is available for two years, in the first instance, and would be of interest to either recent Ph.D. holders or fellows with some years experience.

Starting salary up to £14,703 on the RA1A scale: £10,458–£16,665.

If you wish to have an informal talk about these positions, telephone Dr G H Dodd on 0203 523234.

Application forms from the Personnel Office, University of Warwick, Coventry CV4 7AL (0203 523627) quoting Ref No 3/2A/89/14 (please mark clearly on envelope). Closing date for applications 14 September 1989.

An equal opportunities employer (1420)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL
INSTITUTE FOR GRASSLAND AND ANIMAL PROTECTION
BONE BIOLOGIST: SKELETAL DISEASE IN POULTRY

Bone Biologist, required to play a leading role in a programme of research concerned with skeletal disorders in poultry. The post is based at the Roslin, Edinburgh Station where research on the avian skeleton is being given a very high priority.

The initial and major responsibility of the appointee will be to establish and maintain expertise in orthopaedic clinical diagnosis and bone pathology in relation to the overall programme. The appointee will join a multidisciplinary team of nutritionists, biochemists and physiologists and will be expected to make a major contribution to the experimental research programme. The aim of the research is to reduce the incidence of skeletal disorders in avian species and to maintain a strong science base for developments in this area.

Applications are invited from candidates preferably with a veterinary qualification, although other related disciplines will be considered. Post-graduate experience in bone biology, skeletal development or disease or in pathology would all be relevant. It is probable that the successful applicant will have had some appropriate research experience.

Salary: £13,254-£21,633 according to qualifications and experience.

Applications with the names of three referees to the Head of Division, Poultry Department, Institute for Grassland and Animal Production, Roslin, Midlothian, EH25 9PS quoting E/F/1.

An equal opportunities employer. (1424)A

POSTDOCTORAL RESEARCH ASSOCIATE

Position available in Biochemistry Department, University of Maryland, Dental School. Ph.D. with a strong background in Developmental Biology required. Experience in enzymology, mammalian tissue culture and molecular biology preferred. Salary commensurate with experience.

Submit Curriculum Vitae with three references to: **Professor Y.F. Chang, 666 W. Baltimore Street, Baltimore, MD 21201.** (NW4107)A

SENIOR RESEARCH SCIENTIST

The Wistar Institute is seeking an individual to establish transgenic animal models for the study of rabies virus components and other viral disease of importance to veterinary and human medicine. Qualified candidates should have an MD, PhD or VMD Degree and should possess a minimum of 10 years' experience in experimental mammalian embryology, and proven ability to generate transgenic animals in a variety of species. Additional experience must include an extensive background in virology, embryo-virus interactions and molecular biology.

Submit curriculum vitae to: **Dr. Warren B. Cheston, Associate Director, THE WISTAR INSTITUTE, 36th & Spruce Streets, Philadelphia, PA 19104.** Equal Opportunity Employer, M/F. (NW4105)A

Department of Biological Sciences
University of South Carolina

Faculty Position in Plant Biology

Applications are sought for a tenure-track position available in 1990. We are especially interested in individuals applying molecular and biochemical techniques to questions in plant biology, but the specific field of research is open. Applicants must demonstrate a productive, independent research program. Generous startup funds will be provided. Academic rank is open. Please send curriculum vitae, description of research interests and letters from three references by October 15, 1989 to: **Dr. David Lincoln, Plant Biology Search Committee, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208.** *The University of South Carolina is an Equal Opportunity/Affirmative Action Employer.* (NW4099)A

Assistant Research Molecular Biologist

Department of Biochemistry and Biophysics
University of California, San Francisco

To carry out a broad-based phylogenetic study of small nuclear RNAs in a variety of eurkaryotic organisms. The ARMB will also be in charge of the management of the laboratory, including purchasing and budgeting responsibilities, and supervision and training of laboratory assistants. Research duties include nucleic acid isolation, amplification of RNA using PCR-technology, sequence analysis and computer-assisted determination of RNA secondary structure. Starting salary is $37,700. Requirements: A Ph.D. in Biochemistry, with 3 years of postdoctoral training, demonstrated expertise in DNA cloning, DNA and RNA sequencing, and experience in PCR-based methods, with prior experience in laboratory management. Send cv with the names and telephone numbers of 3 references by 15 September 1989 to: **Dr. Christine Guthrie, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143–0448.** *Equal Opportunity/Affirmative Action Employer.* (NW4108)A

POSTDOCTORAL RESEARCH POSITION

MOLECULAR BIOLOGY AND GENE EXPRESSION

NCI funded position to study hamster glutathione s-transferase gene. Gene is regulated in hamster smooth muscle tumors by glucocorticoids and is of the secondary response class. Experience in molecular biology essential. Position is for a minimum 2 years with starting salary $20,000 and is available September 1. Send letter of application, c.v., and name, address and phone number of 3 references to: **James S. Norris, Ph.D., Professor of Medicine/Cell Biology/Anatomy, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425.**
EO/AA Employer (NW4103)A

THE UNIVERSITY OF THE WEST INDIES

St Augustine, Trinidad

SENIOR LECTURER/ LECTURER IN BIOCHEMISTRY

The appointee should have a higher degree and teaching and research experience in Biochemistry, Microbiology and/or Plant Biochemistry. Experience in gene technology desirable. Annual salary ranges: Senior Lecturer: TT$69,180-TT$90,168; Lecturer: TT$54,708-TT$77,604. Pension, Passages, Housing. Applications detailing qualifications and experience naming three referees to the Registrar, University of the West Indies, St. Augustine, Trinidad. Further particulars sent to all applicants; these are also available from Appointments (36771), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF. (W6431)A

Cancer Research Campaign Technology Ltd

BUSINESS MANAGER

Cancer Research Campaign Technology, a company wholly owned by the Cancer Research Campaign has a vacancy for a Business Manager. The individual appointed will assist in the exploitation and commercialisation of scientific and clinical developments arising from CRC-sponsored research.

Applicants should have a basic training in the medical or biological sciences (to at least first degree level) and several years experience in post-graduate research or industrial, legal or business experience relevant to technology transfer.

The appointment will be within the salary scale £13,000–£16,000 (currently under review, but inclusive of London Weighting). There is a non-contributory pension scheme.

Applications, including a curriculum vitae and the names and address of two referees should be sent by 15 September 1989 to the Personnel Assistant, Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR.

Cancer Research Campaign
fighting cancer on all fronts (1425)A

UNIVERSITY OF NEWCASTLE UPON TYNE
Department of Chemistry

3 YEAR POST-DOCTORAL RESEARCH ASSOCIATE
COMPUTER GRAPHICS AND MOLECULAR RECOGNITION

Applications are invited for the above post for research using new advanced molecular graphics equipment funded by SERC. Work will involve graphics applications in a wide range of molecular recognition fields in chemistry and biochemistry, and possibly associated X-ray crystallographic studies, under the direction of Dr W Clegg. Candidates should have a Ph.D in Chemistry or a related subject; experience in computer graphics and/or crystallography would be an advantage, but provision for appropriate training will be made.

The post is available from 1st October 1989 or as soon as possible thereafter. The salary will be up to £11,088 on the Grade 1 A salary scale.

Applications, including a curriculum vitae and the names of three referees, should be sent as soon as possible to Professor A G Sykes, Department of Chemistry, The University, Newcastle upon Tyne, NE1 7RU. (1411)A

UNIVERSITY OF OXFORD

INSTITUTE OF MOLECULAR MEDICINE
ASTHMA GENETICS GROUP
One Postdoc (1A) and Two Graduate Research Assistants (1B) (Molecular Biology)

Applications are sought for the above posts to join an exciting research programme to characterise the genetic causes of asthma and respiratory allergy. Funding is on a Programme Grant from the Wellcome Trust for 3 years.

Experience in cDNA work would be a definite advantage for the postdoctoral position, and knowledge of gene mapping techniques of help for the graduate research assistants.

Salary: RS1A: £13,527–£16,014 and RS1B: £9,816–£12,381.

The University is an equal opportunity employer.

Applications in writing, together with CV and names and addresses of two referees, quoting ref: GTW/1A/Hopkin for the RS1A post and ref: GTW/1B/Hopkin for RS1B posts, should be sent to:

The Departmental Administrator
Nuffield Dept. of Clinical Medicine
Room 5801
John Radcliffe Hospital
HEADINGTON
OXFORD OX3 9DU

Closing date: two weeks from appearance of advert. (1423)A

AFRC INSTITUTE FOR ANIMAL HEALTH PIRBRIGHT LABORATORY

MOLECULAR BIOLOGIST/VIROLOGIST (SENIOR SCIENTIFIC OFFICER) IMMUNOLOGIST (HIGHER SCIENTIFIC OFFICER)

to work on

MOLECULAR BIOLOGY AND IMMUNOLOGY OF RINDERPEST VIRUS — RESEARCH PROJECT FUNDED BY THE WELLCOME TRUST

Rinderpest virus is a serious veterinary pathogen, closely related to measles and canine distemper virus, which affects ruminants in many developing countries. It is an excellent model for the study of virus pathogenicity in the natural host at the molecular level since strains differing greatly in their virulence have been isolated. For the senior position (SSO), we are looking for a scientist who is interested in the analysis of the structure and function of virus proteins using molecular biological techniques. For the junior position (HSO), we are looking for a post-doctoral scientist interested in the immunology of virus infections. SSO position for approximately 5 years and the HSO position for 3 years.

Qualifications:
First or second class honours degree in appropriate subject. Ph.D. preferred for both posts.

Experience:
For SSO appointment a minimum of four years' post-graduate research experience is essential. For HSO, candidates must offer at least two years' post-graduate experience directly relevant to the post.

Salary:
SSO £12,445–£17,032
HSO £10,026–£13,460
PAY AWARD PENDING

Annual Leave:
25 days plus 10½ days public and privilege holiday.

Informal enquiries can be made by phone **(0483 232441)** to **Dr T. Barrett** or letter to **Dr C.J. Bostock, Head of Molecular Biology.** Applications including a full curriculum vitae and the names of two referees should be sent to:

Laboratory Administrator, AFRC Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey GU24 0NF

Closing date: 14th September 1989

AFRC is an equal opportunity employer. (1410)A

MEDICAL RESEARCH COUNCIL

MRC COLLABORATIVE CENTRE/NATIONAL INSTITUTE FOR MEDICAL RESEARCH

POSTDOCTORAL BIOCHEMISTS/ MOLECULAR PHARMACOLOGISTS/ CHEMISTS

Applications are invited from postdoctoral scientists for two short-term (3 year) scientific staff appointments to do research on the control of muscarinic receptor — G protein coupling and the mechanism of activation by acetylcholine. One scientist should have a training in organic chemistry/medicinal chemistry and the other in biochemistry/molecular pharmacology. The project will form an integral part of the investigation by the muscarinic receptor group (Drs N J M Birdsall, N J Buckley and E C Hulme, Division of Physical Biochemistry, NIMR) of the structure, function and regulation of muscarinic acetylcholine receptors and their genes.

The salary will be in the range £11,070-£19,310 plus £1,650 per annum London Allowance (pay award pending). MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees, should be sent to **Mr C R Russell, Administrative Manager, NIMR, The Ridgeway, Mill Hill, London NW7 1AA not later than 15th September 1989** *quoting reference number: CCPB/2.*

The MRC is an Equal Opportunities Employer. (1392)A

MRC

FELLOWSHIPS

NATIONAL CANCER INSTITUTE–FREDERICK CANCER RESEARCH FACILITY
BRI–Basic Research Program
POSTDOCTORAL FELLOWSHIPS

BRI–Basic Research Program Postdoctoral Fellowships are awarded for 1–3 years with stipends ranging from $24,000 to $30,000/year. The senior staff members and their research interests are

George Vande Woude: molecular basis of neoplastic transformation

Stephen Hughes: structure and function of HIV reverse transcriptase and integration protein; expression of cytoskeletal genes; retroviral vectors; transgenic birds

George Pavlakis: eukaryotic gene regulation; molecular mechanisms of cell transformation; molecular biology of HIV and pathogenesis of AIDS

Peter Johnson: mammalian transcription factors; molecular basis of tissue–specific gene expression

Stephen Oroszlan: immunochemistry and protein chemistry of retroviruses; structure and function of retroviral gene products; proteases

Alan Rein: retroviral genetics; functional analysis of retroviral genes using natural and synthetic mutants

Nancy Rice: molecular biology of retroviruses; oncogene expression

William Lijinsky: environmental carcinogenesis; mechanisms of carcinogenesis by nitrosamines and related compounds

Anthony Dipple: carcinogen–DNA interactions; polycyclic aromatic hydrocarbon carcinogenesis and mutagenesis

Robert Moschel: chemical synthesis of carcinogen–modified DNA; physical chemistry of carcinogen–DNA interactions; DNA adduct–induced mutagenesis in bacteria and mammalian cells

Christopher Michejda: biochemistry of carcinogen activation; DNA alkylation; chemotherapeutic agents

Jeffrey Strathern: genome rearrangement, recombination, and DNA repair in yeast; cell type regulation

David Garfinkel: molecular biology of the retrotransposon Ty1; insertional mutagenesis by Ty; gene regulation

Amar Klar: gene regulation and homothallic mating–type switching in fission and budding yeast

Stuart Austin: chromosome stability in bacteria: regulation of plasmid replication and distribution of copies to daughter cells

Richard Fishel: molecular mechanisms and biochemistry of genetic recombination in mammalian cells

Donald Court: regulation of gene expression by transcription initiation, transcription termination, and RNA processing

Neal Copeland: molecular genetics of murine leukemogenesis; developmental genetics; transposable elements and retroviruses

Nancy Jenkins: insertional mutagenesis by retroviral DNAs; transgenic mice; molecular biology of mouse development

Luis F. Parada: use of ES cells to study molecular biology of development, the role of oncogenes, and targeted homologous recombination; *in situ* hybridization analysis of development

Peter Donovan: development of the mouse germ line; germ cell gene expression; germ line mutations; cell adhesion molecules

Alexander Wlodawer: structure of enzymes and oncogene products studied by X–ray diffraction

Irene Weber: crystallographic analysis of proteins and nucleic acids; modeling of unknown protein structures; protein–ligand interactions

J. Ronald Rubin: crystallographic investigations of ligand–macromolecule interactions and drug–nucleic acid interactions

Send a curriculum vitae and the names of three references to the investigator(s) of interest, c/o Dr. Maurice L. Guss, BRI–Basic Research Program, NCI–Frederick Cancer Research Facility, P.O. Box B (Rm 48), Frederick, Maryland 21701.

An equal opportunity/affirmative action employer M/F/H/V (NW4085)E

THE UNIVERSITY OF BIRMINGHAM

SCHOOL OF BIOLOGICAL SCIENCES

Post-doctoral Fellowships

AFRC PLANT MOLECULAR BIOLOGY INITIATIVE

The School of Biological Sciences at the University of Birmingham has received several grants under this initiative and applications are now invited for the following post-doctoral positions:-

(1) Host control of gene expression in the wheat powdery mildew pathogen (*Erysiphe graminis f.sp. tritici*) Dr. C. E. Caten and Dr. D. W. Hollomon, (IACR-LARS). Ref. No. 2168.

The project will investigate the role of host signals in parasitism of plants by obligate biotrophic fungi. This will involve transferring genes and expression sequences from the wheat powdery mildew fungus into the facultative pathogen, *Septoria nodorum*, and assaying their functioning both in culture and on the host plant. The project is in collaboration with the Crop Protection Department, IACR – Long Ashton Research Station, Bristol. Applicants should have research experience in molecular biology, genetics, biochemistry, physiological or molecular plant pathology.

(2) The molecular biology of the synaptonemal complex (SC) in meiotic nuclei of tomato (Drs. G. H. Jones, F. C. H. Franklin and J. S. Parker (QMC, London)). Ref. No. 2169.

The aim of this project is to identify and characterise protein components of the synaptonemal complex, and the genes encoding them. This will involve isolation and purification of synaptonemal complexes from meiotic prophase I nuclei, production of anti-synaptonemal complex antibodies and the cloning and characterisation of synaptonemal complex genes. Applicants should ideally have some experience of antibody technology, but persons with a general background in molecular biology or cytology will be considered.

(3) Cell-Cell Recognition in Plants: The Molecular Biology of Self-Incompatibility. (Drs. F. C. H. Franklin and M. J. Lawrence). Ref. No. 2170.

The aims of the project are to continue the characterisation of the stigmatic S-glycoprotein that we have recently identified and in clone the gene that encodes it, and to identify the pollen receptor with which it interacts. Additionally, we intend to clone and characterise the *Brassica* S-gene homologues that have been discovered in *P.rhoeas*. Applicants should ideally have experience in molecular biology or biochemistry.

(4) Cell-Cell Recognition in Plants: Cell Surface Antigens and Gamete Receptors (Professor J. A. Callow and Dr. J. R. Green). Ref. No. 2171.

Recent studies in the supervisors' laboratory have used a monoclonal antibody approach to characterise surface antigens and receptors potentially involved in the egg-sperm interaction of *Fucus*, a good model system for cellular and molecular recognition studies. This project will consolidate this approach and will involve generating further monoclonal antibodies to gamete surface glycoproteins, the isolation of these glycoproteins and their biochemical characterisation. Applicants should ideally possess a background in cell biology (animal or plant), with experience in working with antibodies, membranes or glycoproteins, but good applicants with general training in biochemistry, carbohydrate chemistry or a relevant biological discipline will be considered.

All appointments will be for three years with starting dates between 1st October 1989 and 1st March 1990. Further information can be obtained informally by contacting the project supervisors (School telephone number 021-414 5923).

Application forms are available from the Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham, B15 2TT or by telephoning 021-414 6483 (24 hour answerphone). Please quote the appropriate reference number. Closing date: 15th September 1989.

An Equal Opportunities Employer. 391)E

UNIVERSITY OF EDINBURGH
HIV IMMUNOLOGY UNIT
DEPARTMENT OF MEDICINE

POST DOCTORAL FELLOWSHIP

Applications are invited from suitably qualified people with an interest in HIV to work on an MRC funded project on cytotoxic T cells in HIV infected individuals. A good background in Immunology and experience in cell culture would be an advantage. The salary will be at the appropriate point on scale 1A (£10485-£16665). The position is funded for 3 years by the MRC and is available immediately.

For further information, please contact **Dr. Graham Bird or Dr. Karin Froebel at the HIV Immunology Unit, Dept. Medicine, Royal Infirmary, Edinburgh EH3 9HB**, tel: 031.229.2585 ext. 235.

Applications should include a curriculum vitae and the names of 2 referees and should be sent to Dr. Bird by 8th September, 1989.

Please quote reference no. 5698 (1394)E

UNIVERSITY OF ABERDEEN
DEPARTMENT OF GEOLOGY AND PETROLEUM GEOLOGY

RESEARCH FELLOWSHIP IN IGNEOUS PETROLOGY

This Research Fellowship, which will involve some teaching duties, is in the field of Igneous Petrology. The Department has a strong research group working on metalliferous economic geology and an interest in this area may be an advantage. The appointment will be for five years and is available from 1 October 1989. Salary will be within the range £10458-£16665 on the IA (postdoctoral) Scale for Research and Analogous Staff.

Further particulars and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX (tel 0224 273500)** to whom applications (2 copies) should be returned by 15 September 1989 quoting ref no LW/045. (1397)E

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

The Japanese Science and Technology Agency (STA) is offering postdoctoral fellowships to scientists and engineers of the countries listed below for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

The fellowships are open to young PhD holders of under 35 (although older researchers will be considered) from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of invitation from their Japanese host institution (the organizations listed in Table 1 provide help in contacting suitable host institutions).
Those in the final stages of a PhD may also apply.

There are no closing dates but candidates are encouraged to submit their applications as soon as possible. Fellowships for fiscal 1989 must be taken up by March 1990.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class) and the following tax-free allowances:

1) Living allowance: ¥270,000 (about US$2,000) a month
2) Family allowance: ¥ 50,000 a month
3) Housing allowance: up to ¥100,000 a month
   Apartments will normally be provided to awardees. The apartment floor area is 40 m^2 for awardees unaccompanied by their family and 60 m^2 for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) International relocation allowance: ¥200,000
5) Travel allowance: (within Japan) ¥100,000 a year
6) Japanese language lesson courses are to be provided free of charge to the STA Fellowship awardees and their family members in Tsukuba area. Those who live in places other than the Tsukuba area will be entitled to reimbursement of Japanese language school tuition up to a specified amount.
7) Excursions or the like will be held to help make the Fellowship awardees and their family members in Tokyo/Tsukuba areas familiarized with Japan's culture, tradition and history.

In addition, ¥1,480,000 per year will be paid to the host institute to cover research costs and insurance for researchers will be paid by JISTEC during their stay in Japan to cover medical care.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Management of the STA Fellowship, including recruitment of candidates, is entrusted to the Japan International Science and Technology Exchange Center (JISTEC).

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country. Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

A researcher whose country is not listed in Table 1 could contact a Japanese host institute directly, which in turn may recommend the reseacher to JISTEC as a candidate for STA Fellowship.

Fig. 1 shows the process from consult/contact to receipt of award.

(W6352)E

Fig. 1 PROCESS FLOW FROM CONSULT/CONTACT TO RECEIPT OF AWARD

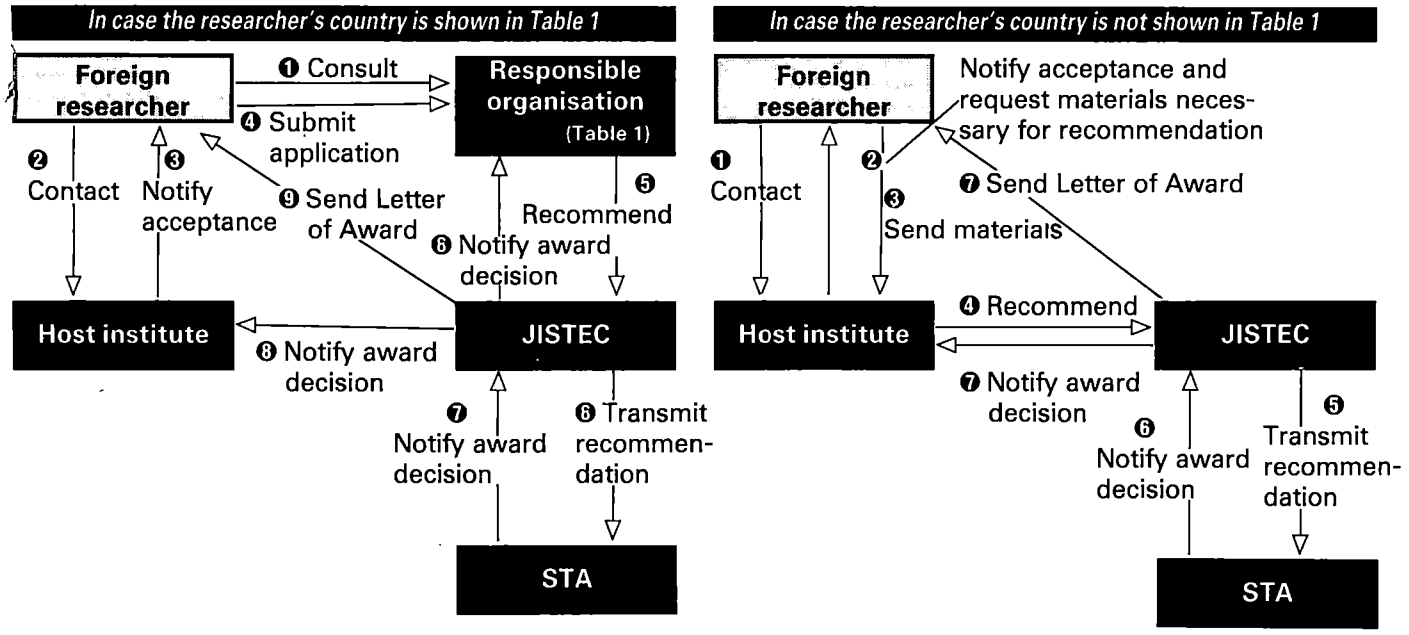


Table 1 RESPONSIBLE ORGANIZATIONS

Country	Contact
Australia	DEPARTMENT OF INDUSTRY, TECHNOLOGY AND COMMERCE The Secretary (Attention: Assistant Secretary, Japan Branch) GPO Box 9839, Canberra ACT 2601 Tel: 062-76-1000 Fax: 062-76-1122
Canada	NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL Dr. R.J. Kavanagh Director-General (Scholarships & International Programs) Centennial Towers 200 Kent St., Ottawa, Ontario K1A 1H5
Federal Republic of Germany	ALEXANDER VON HUMBOLDT-STIFTUNG Dr. Rolf Hoffmann Selection Department Jean-Paul-Strasse 12 5300 Bonn 2, FRG Tel: (0228) 833-0 Fax: (0228 833-199
Finland	MINISTRY OF TRADE AND INDUSTRY Division for International Affairs Mr. Pertti Valtonen Head of Division Aleksanterinkatu 10, SF-00170 Helsinki
France	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE Mr. Stuyck Taillandier Direction des Relations et de la Cooperation Internationales 15 Quai Anatole France, 75700 Paris Tel: 1-47 53 1515
Italy	MINISTERO DELLA RICERCA SCIENTIFICA E TECNOLOGICA Ufficio Relazioni Internazionali Dr. Mario Bove Director Lungotevere Thaon di Revel 76, 00100 Rome Tel: 6-369-941 Fax: 6-392-209 Tlx: 612548 RISCIE 1
Netherlands	MINISTRY OF EDUCATION AND SCIENCE Dr. ir. B. Okkerse Director-General for Higher Education and Scientific Research Dr. P. van't Klooster Deputy Director Division Research Organisations Directorate-General for Higher Education and Scientific Research P.O. Box 25000, 2700 LZ Zoetemeer
New Zealand	INTERNATIONAL SCIENCE UNIT Dept. of Scientific & Industrial Research Mr. M.A. Collins Assistant Director-General P.O. Box 1578, Wellington
Sweden	STYRELSEN FOR TEKNISK UTVECKLING Dr. Erik von Bahr Box 43200, 100 72 Stockholm Tel: 08-775 40 00 Fax: 19 68 26 Tlx: 10840 swedstu s
Switzerland	SWISS NATIONAL SCIENCE FOUNDATION Mr. Benno Frey Wildhainweg 20, CH-3001 Bern Tel: 031-24-54-24 Fax: 23-30-09 Tlx: 912-423
United Kingdom	THE ROYAL SOCIETY Ms. Karen Kimpton or Dr. Stephen Cox 6 Carlton House Terrace, London SW1Y 5AG Tel: 01-839-5561 Tlx: 917876
United States	NATIONAL SCIENCE FOUNDATION Dr. Charles W. Wallace Senior Program Manager US-Japan, Australia and New Zealand Programs Division of International Programs Washington, D.C. 20550 Tel: 202-357-9558
European Communities	THE COMMISSION OF THE EUROPEAN COMMUNITIES Mr. Giorgio Boggio, DG XII Head of Division, Rue de la Loi 200, 1049 Brussels Tel: 235-5635

QUESTIONS ABOUT STA FELLOWSHIP

Please direct questions about the fellowship scheme to the responsible organization in your country given in Table 1. If your country is not listed, inquiries will be received by JISTEC.

Japan International Science and Technology Exchange Center (JISTEC)

Address: Port One Building 6F, 1-7-6, Minato-machi
Tsuchiura City, Ibaraki Pref. 300
Japan
Telephone: 0298-24-3355
Facsimile: 0298-24-3214

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology and Medicine)

(University of London)

Norfolk Place, LONDON W2 1PG

DEPARTMENT OF BIOCHEMISTRY & MEDICAL GENETICS

MRC FUNDED PhD STUDENTSHIP

An MRC PhD studentship is available from 1 October under the Human Genome Mapping Project initiative to work on the physical mapping of the region around the malignant hyperthermia susceptibility locus. A recent graduate with a keen interest in human genetic disorders is sought. Applicants for this post should contact **Dr. Keith Johnson at the above address,** or by telephone for further details (01) 723-1252 Ext. 5480. Include with your application a C.V. and names and details of 2 referees. Closing date 8 September. (1402)F

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF READING
and
INSTITUTE OF FOOD RESEARCH
READING LABORATORY

Protein Engineering and Gene Expression AFRC-CASE PhD Studentship

Applications are invited for an AFRC-CASE Studentship, leading to a PhD in the area of molecular genetics. The project will involve the protein engineering, expression and secretion of commercially important enzymes from the filamentous fungus *Neurospora crassa*. Applicants should have or expect to obtain a first or upper second class Honours degree in a Life Science. For further details contact Dr. I. Connerton (0734-318895).

Application with c.v. and names of two academic referees to: **Dr. I. Connerton, Department of Microbiology, University of Reading, London Road, Reading RG1 5AQ.** (1371)F

SCOTTISH CROP RESEARCH INSTITUTE
Invergowrie, Dundee

Ph.D. STUDENTSHIP TISSUE CULTURE DEPARTMENT

Applications are invited for a Ph.D. Studentship funded by the Biscuit, Cake and Confectionery Alliance and will involve research in the use of protein and DNA markers to genetically fingerprint cocoa (*Theobrome cacao L.*) germplasm.

The primary objectives of the proposal are twofold. First to use available isozyme and RFLP technology to genetically fingerprint cocoa germplasm. Rapid and reliable "user friendly" methods will be developed to differentiate between cocoa genotypes. Second, to use this information to estimate allelic diversity within the cocoa gene pool. It is anticipated that the methods developed will provide powerful tools for exploitation in various aspects of cocoa improvement.

The Studentship will commence on 1 October 1989 and the stipend will be £4,000 per annum rising to £5,000 per annum in the third year. Applicants should have a first class or upper second class honours degree or equivalent experience. Interested applicants are invited to contact W. Powell/ B.P. Forster/R. Waugh, Tissue Culture Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA. Tel. (0382) 562731. (1421)F

AFRC INSTITUTE OF ANIMAL PHYSIOLOGY AND GENETICS RESEARCH

RESEARCH STUDENTSHIP

CAMBRIDGE

Olfactory Binding Proteins

Applications are invited for an industrially funded studentship to support research leading to a PhD in the University of Cambridge. The project, to be supervised by Dr W D Booth, will involve study of odorant binding proteins in the nasal mucosa of the pig with particular reference to musk-odour compounds and the possible dependence of odour binding phenomena on endocrine status.

Applicants should hold a 1st or upper 2nd class degree in biochemistry or biological sciences with a bias towards protein biochemistry. Applications including a full curriculum vitae naming two referees should be submitted as soon as possible to Dr R J Bicknell, Tutor for Research Students, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, CB2 4AT. Informal enquiries are welcomed. (Tel. 0223-832312). (1365)F

PROTEIN CRYSTALLOGRAPHER

required for BIOPHYSICS GROUP, IMPERIAL COLLEGE

A postdoctoral Research Assistantship is vacant in an active group of 15 scientists working on protein crystallography, protein crystallisation and protein modelling. We are seeking a trained crystallographer interested in advancing experimental techniques, and in solving and interpreting new structures. Structures currently being studied include collagenase, cholesterol oxidase, xylose isomerase, *Erythrina* trypsin inhibitor and glyceraldehyde-3-phosphate dehydrogenase; proteins actively being crystallised include engineered fragments of tissue plasminogen activator, carboxypeptidase G and the reverse transcriptase of HIV.

The vacant post is associated with our Medical Research Council programme which has long-term support, and will be for work on medically relevant enzymes. The group is well equipped (VAX computers, FAST diffractometer, Evans & Sutherland Picture System), and has a wide network of collaboration with academic and industrial groups worldwide.

Applications including C.V. and names of two referees to **Professor D.M. Blow, Blackett Laboratory, Imperial College, Prince Consort Rd, London SW7 2BZ, UK (01-589 5111 ext 6721)** who can give further information. (1389)P

UNIVERSITY OF SUSSEX
PHYSICS AND ASTRONOMY

LECTURESHIP IN OBSERVATIONAL ASTRONOMY RESEARCH FELLOWSHIP IN OBSERVATIONAL ASTRONOMY

Applications are invited for a lectureship in observational astronomy. The appointment will be made as soon as possible but University funds to support the lecturer will not be available earlier than October 1993. Applicants should either currently possess a fellowship with at least four years to run, which could be tenable at the University of Sussex, or be candidates for the research fellowship in observational astronomy advertised with this lectureship or be applicants for an SERC Advanced Fellowship from October 1990 tenable at the University of Sussex.

Applications are also invited for an SERC supported research fellowship in observational astronomy tenable for up to three years. Candidates should work either with Dr R C Smith on cataclysmic variable stars or with Dr A C Cameron on the solar stellar connection. Candidates may also, if they wish, be considered for the lectureship in observational astronomy advertised with this fellowship. In that case the tenure may be for up to four years and other fields of research which relate to the theoretical work of the Astronomy Centre would be acceptable.

Applications for both posts should be addressed to **Professor R J Tayler, School of Mathematical and Physical Sciences, Physics Building, University of Sussex, Falmer, Brighton BN1 9QH,** from whom further particulars can be obtained and should arrive not later than October 31st 1989. However note that the closing date for applications for SERC Advanced Fellowships is September 30th 1989. The starting salary for the research fellowship is unlikely to be higher than £12,879 on the Research 1A scale.

AN EQUAL OPPORTUNITY EMPLOYER (1405)E

POSTDOCTORAL FELLOWSHIPS

in gastroenterological research available for two or more years beginning 1990. Faculty employs multidisciplinary approach using biochemical, cell/molecular biological, immunological, physiological and electrophysiological techniques. Applicant may focus on clinical and/or basic studies. Applicants should have M.D. or Ph.D. Send curriculum vitae and names of three references to **N. F. LaRusso, M.D., Program Director, NIH Training Grant, Mayo Clinic, Rochester, MN 55905.** An equal opportunity/ affirmative action employer. (NW4101)E

TRANSMEMBRANE SIGNALLING

January 4th-6th, 1990

University of Liverpool, U.K.

INVITED SPEAKERS AND CHAIRMEN INCLUDE:

P. Andersen, P. Ascher, E. Barnard, M.J. Berridge, D.A. Brown, R.D. Burgoyne, T. Cheek, P. Cobbold, S. Dissing, J. Garthwaite, B. Gomperts, M. Houslay, R.F. Irvine, M. Lazdunski, O.H. Petersen, B. Sakmann, I. Schulz, C.B. Wollheim.

SESSIONS WILL COVER:

Ca^{2+} oscillations; Ca^{2+} stores; inositol polyphosphates; protein kinase C; agonist-operated ion channels; K^+ channels; neurotoxins; G-proteins; NMDA receptors; Long-term-potentiation; exocytosis.

FREE COMMUNICATIONS (10 min.)

The Symposium is held in association with a national scientific meeting of The Physiological Society in Liverpool. Deadline for submission of abstracts is 1st October, 1989.

REGISTRATION AND FURTHER INFORMATION:

Mrs. B.A. Fairfoull, MRC Secretory Control Research Group, The Physiological Laboratory, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, U.K. FAX: 44 51 794 5327; Telephone: 44 51 794 5322.

Cost of registration: £30.00 (except for members of The Physiological Society). (1393)C

Royal Postgraduate Medical School

(University of London)

Modern Techniques in Cell Biology and Pathology

4–8th December 1989

This one week course is aimed to give an understanding of the practical and theoretical bases for using modern techniques in the future of pathology and in cell biology including methods to avoid the use of animals. Thus, materials such as fine-needle aspiration biopsies and cell cultures will be considered, with techniques suitable for light and electron microscopy. Topics to be covered include primary and established cell cultures, changes occurring in cells during culture and the optimisation of treatment of pathology specimens to yield maximum diagnostic information. Techniques to be considered include immunocytochemistry, antibody production, DNA and RNA hybridisation including *in situ* techniques, receptor/binding site visualisation, *in vitro* pharmacology, methods for quantitation of labelling, morphometry and electron microscopy.

Invited speakers include:

Prof G Burnstock (London)
Dr C Hassall (London)
Dr D McCance (London)
Dr R Penketh (London)
Dr J Beesley (Kent)
Dr F Ramaekers (Holland)
Dt T Krausz (London)
Dr G Gabella (London)
Dr D Springall (London)

The course would be suitable for scientists or pathologists working with cells in biology or pathology.

Course organisers: Julia M Polak, David Springall (Histochemistry Unit) and Thomas Krausz (Histopathology)

Course fee (including catering) **£350**

Application forms from: Wolfson Conference Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN

Telephone: 01 740 3117

Closing date for applications: 31st October 1989

(1367)C

EC CONCERTED RESEARCH PROGRAMME EUROPEAN VACCINE AGAINST AIDS (EVA)

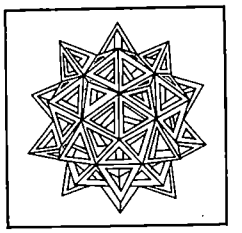
Call for Proposals for Collaboration

The objective of this European programme is to assist and support European efforts towards the development of vaccines against AIDS through the provision of various high quality laboratory reagents for studies leading to vaccine development.

Proposals identifying research reagents needed in vaccine development projects are requested. Those aimed at developing new reagents will also be considered.

The proposals should give scientific reasons for the projects and detailed characteristics that the requested materials should have. Organisations or individuals from EC or COST countries or from those with special arrangements with the EC (Israel/Iceland) wishing to respond to this request should contact **Dr Harvey Holmes, NIBSC, South Mimms, Potters Bar, Herts EN6 3QG, United Kingdom — Tel: +44 707 54753, Fax: +44 707 46730.**

Application deadline is the 31st October 1989. (1295)X



FONDAZIONE SIGMA-TAU

KRAEPELIN'S WORKSHOP

16-17 september 1989

Grand Hotel Des Iles Borromées
Stresa (Novara) - Italy

The renewed interest in the classification of illness in the sphere of neuropsychiatry has stimulated a rediscovery of the work of Emil Kraepelin. By the definition of dementia praecox and manic depressive psycosis, Kraepelin's work has guided the development of neuropsychiatry through its ramifications. Beginning this year and in September of each year thereafter, Foundation Sigma-Tau will sponsor a meeting in honor of Emil Kraepelin in Stresa (Novara - Italy) where he owned a house to which he retreated for his studies.
The theme of the 1st Emil Kraepelin Workshop on September 16-17 1989, is "DEMENTIA".

Saturday 16th september 1989
10 am - 12.30 pm

THE INFLUENCE OF EMIL KRAEPELIN ON CLINICAL NEUROPSYCHIATRY

Chairman:
L. Ravizza (Torino)

Discussant:
Sir M. Roth (Cambridge), V. Conde Lopez (Villadolid), C.L. Cazzullo (Milano), H. Hippius (Munich), T.A. Ban (Nashville).

3 - 6 pm
"WHAT DEMENTIA MEANS TO THE SPECIALIST?"

Chairman:
G. Baumgartner (Zurich)

Discussant:
C.G. Gottfries (Gothenburg):
"Dementia: the neurochemists viewpoint"
V. Chan Palay (Zurich):
"Dementia: a disease of neuronal communication breakdown in neurotransmitter systems"
A. Oliverio (Roma):
"A neurophysiological and pharmacological approach to Dementia"
H. Hippius (Munich):
"Dementia: from the psychiatrists standpoint"
J.M. Martinez-Lage (Pamplona):
"Early Diagnosis in Dementia"
K.L. Leender (Villigen):
"PET studies in Dementia: present findings and future perspectives"

Sunday, 17th september 1989
10 am - 12.30 pm

Chairman:
A. Agnoli (Roma)
Presentation of "Memories" by Emil Kraepelin
G. Kantzà and P. Kantzas (eds.)

General discussion (W6436)A

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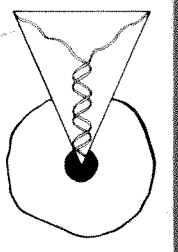

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Eric Lander, Whitehead Institute (rapporteur)

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Leon Rosenberg, Yale (chairman)
Irving Weissman, Stanford University
Richard Mulligan, Whitehead Institute
French Anderson, NIH
David Baltimore, Whitehead (rapporteur)

3 HUMAN GENE RESEARCH: PANEL DISCUSSION

4 TRANSGENIC MOUSE MODELS

Richard Palmiter, Washington U. (chairman)
Douglas Hanahan, University of California SF
Erwin Wagner, IMP Vienna
Ron Evans, Salk Institute
Brigid Hogan, Vanderbilt U. (rapporteur)

5 GENE DISRUPTION AND DEVELOPMENT

Mario Capecchi, U. of Utah (chairman)
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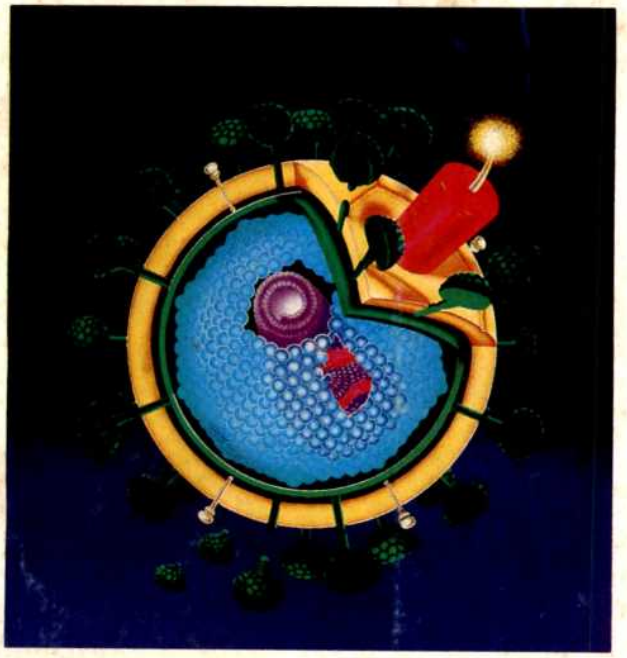

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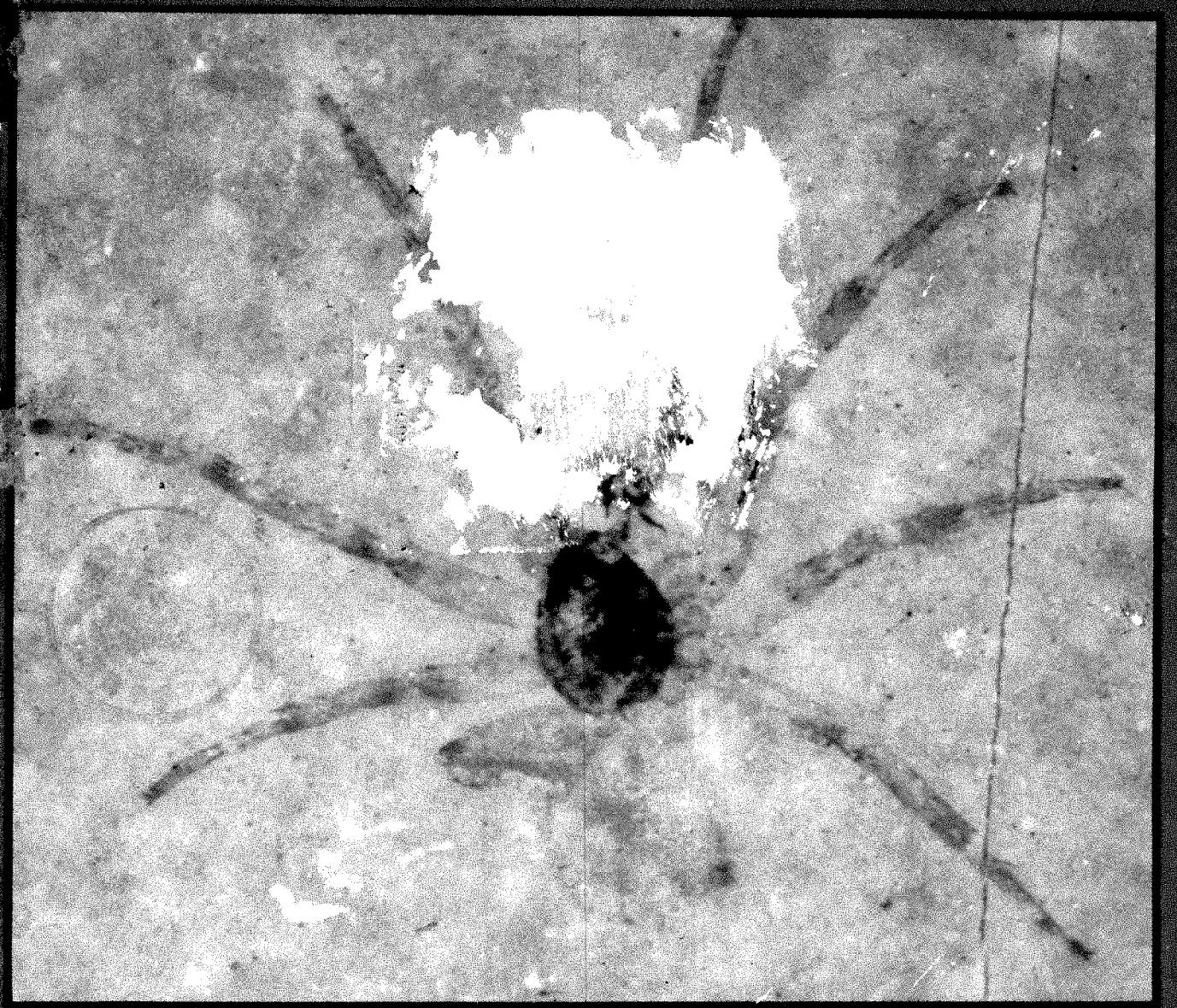

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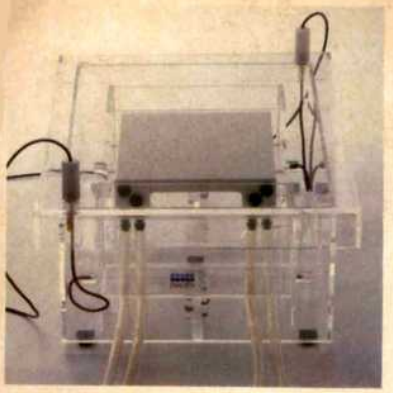

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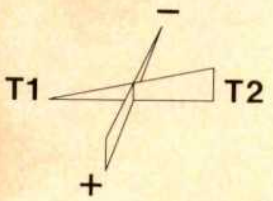

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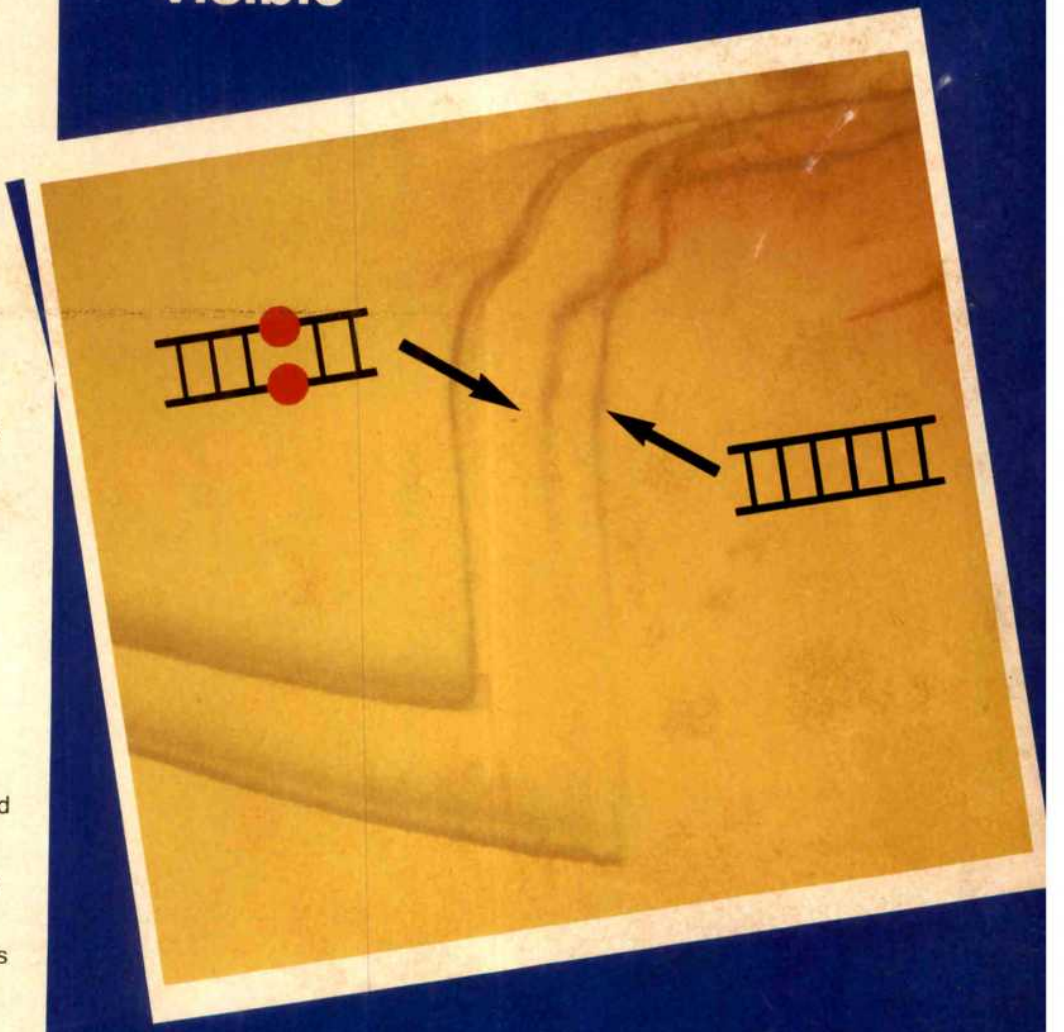

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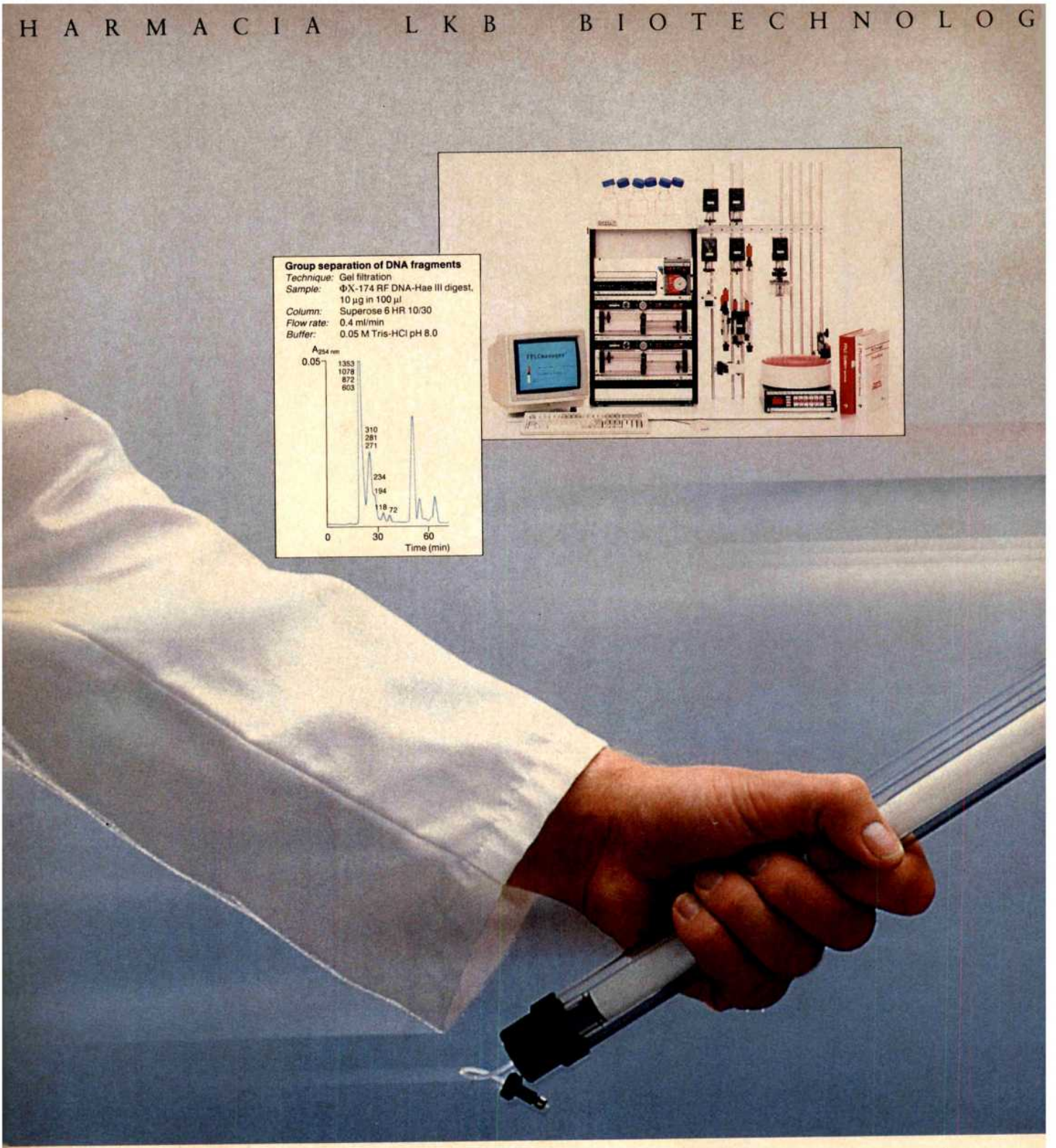

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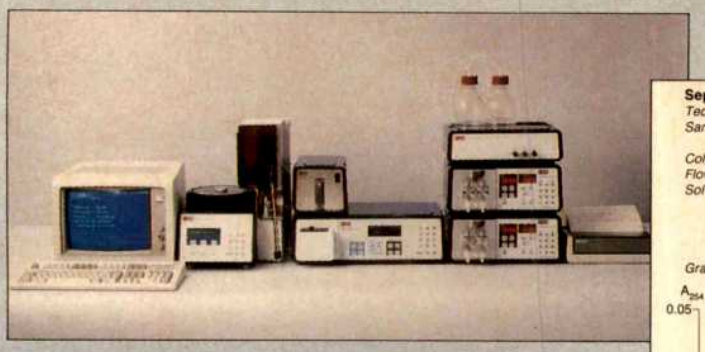

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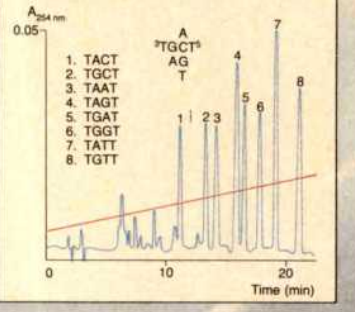

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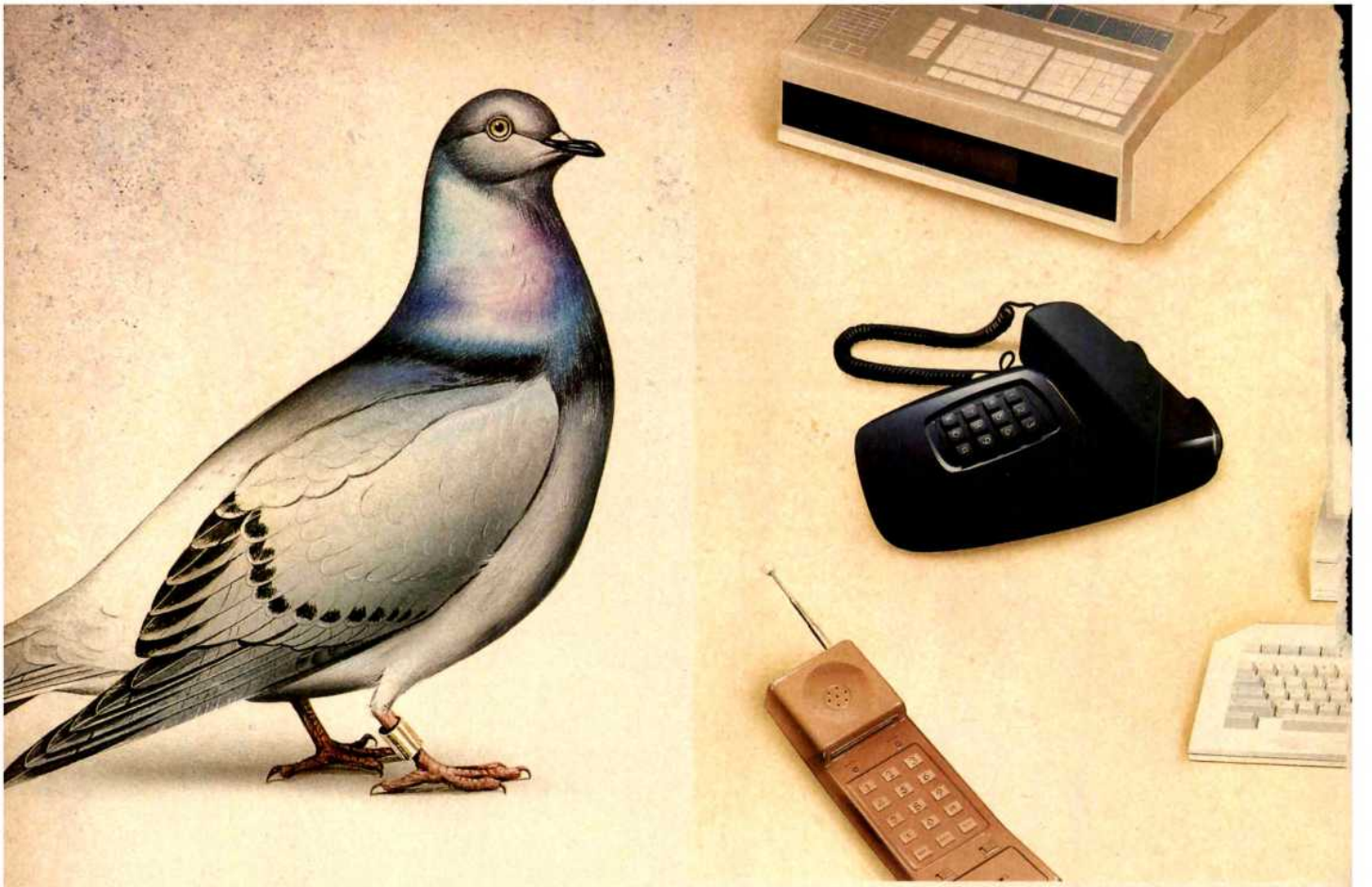

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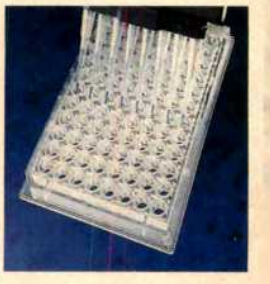

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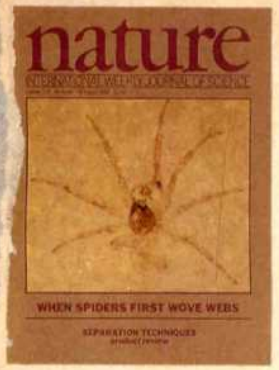

nature

31 August 1989
Vol. 340 Issue no. 6236

◀ Fossil of a mature male web-spinning spider found in Lower Cretaceous lithographic limestone from the Sierra de Montesch in north-east Spain. This represents perhaps the earliest evidence (around 120 million years ago) for the use of woven silk in prey capture. See page 711.

THIS WEEK

Corporate mergers

The 'merging' of smaller galaxies into much larger companions may generate the massive energies required to drive the creation of new stars, formation of black holes and even the evolution of quasars. Pages 687 and 675.

Binding commitment

The first DNA-binding protein to have been found in a higher plant (page 727) shows marked similarities to yeast and animal binding proteins such as CREB, GCN4 and c-Jun — including a 'leucine-zipper' region. This points to an ancient origin, before animals and plants diverged about 1,500 million years ago.

Boundary conditions

Few opportunities arise for examining the fine-scale changes that occurred through and beyond the Cretaceous/Tertiary boundary. But on page 708, Johnson *et al.* report the analysis of a high-resolution record of fossilized plants spanning the boundary. The findings support the contention that there was a climatic warming in the latest Cretaceous, followed by impact with an extra-terrestrial body at the boundary itself.

Enough zed?

Measurements of the Z^0 mass at the Stanford Linear Collider may indicate revisions are needed for the standard theory of electro-weak interactions. But it is early days...Page 677.

Competition policy

Competition between three rock-pool zooplankton species increases local extinction rates and influences the distributional dynamics of the species in the field, as predicted by theories of community ecology, page 713.

On the flat

The structure of *E. coli* RNA polymerase has so far eluded X-ray crystallographers because the protein does not form 3D crystals. Now, though, the formation of 2D crystals of RNA polymerase on a lipid layer has allowed the overall structure of the protein to be determined by electron microscopy. The protein is seen to have a cleft of similar shape to that of DNA polymerase I. Page 730.

New oncogenes due

A subset of human pituitary tumours that secrete growth hormone carries point mutations in the gene encoding the GTP-binding subunit of the stimulatory regulator of adenylyl cyclase, G_s, which result in autonomous cyclic AMP synthesis. This finding leads to the prediction of a new series of oncogenes. Pages 692 and 678.

Breaking with tradition

Tsunami waves generated by sub-sea earthquakes offer an alternative means to traditional seismology for studying fault zones. Page 674.

Affairs of the heart

Calcitonin gene-related peptide transiently stimulates a calcium current in heart muscle that is fundamental to the cardiac pacemaker. This contrasts with the beta-adrenergic agonists which have a sustained effect on this current, page 721. The calcium channel is one of a series known to be modulated by cardiac beta-adrenergic receptors via cAMP-dependent protein kinase activation. On page 718, however, it is reported that beta-adrenergic stimulation also induces large chloride currents that may help to control the action potential.

Guide to Authors

Facing page 732.

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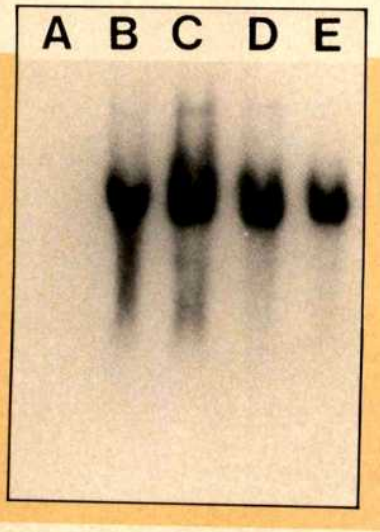

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B: 5 μg transgenic mouse liver total RNA.
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References

1. Aviv, H. and Leader, P. (1972). Proc. Natl. Acad. Sci. 69:1408.

2. Ausubel, F., et al. (1987). Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Interscience.

3. Dycaico, M. and Cramer, K. (1989). Stratagies 2.3:42.

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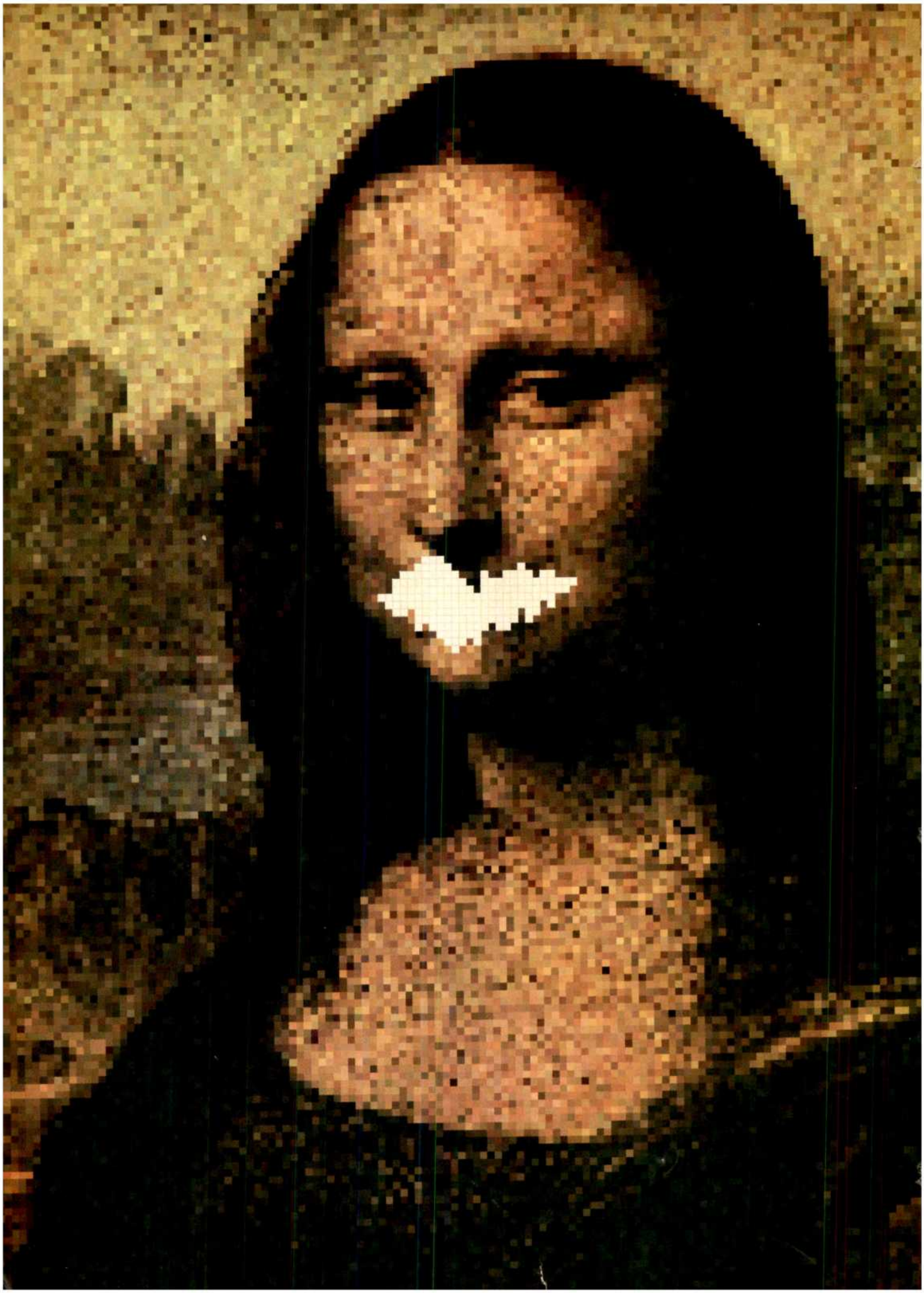

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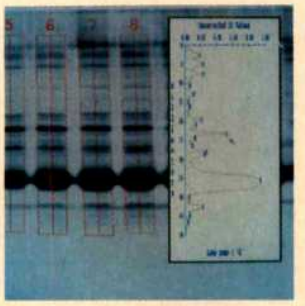
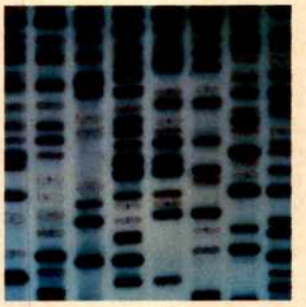
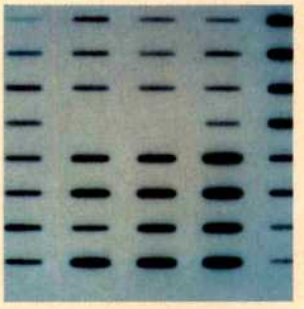


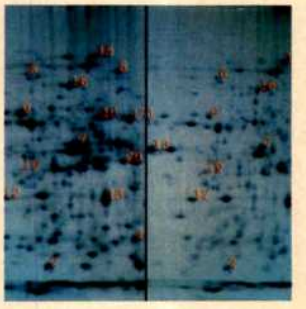
1-D Analysis



Whole Band 1-D Analysis



Blot Analysis



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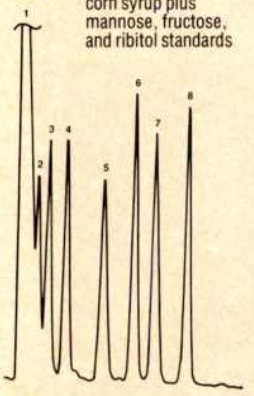
Conditions
Detector: RI Monitor
Column: Aminex CSA
Sample: 2% 35 DE corn syrup plus mannose, fructose, and ribitol standards



Peaks:
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2. Dp 4
3. Dp 3
4. Dp 2
5. Glucose
6. Mannose
7. Fructose
8. Ribitol

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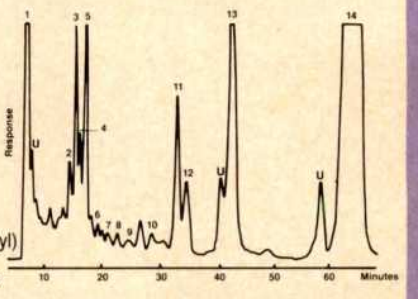
Conditions
Column: Aminex HPX-87H, 300 mm x 7.8 mm
Sample: *P. anaerobius* extract
Detector: UV Monitor @ 210 nm

Peaks:
1. Solvent Peak
2. Lactic
3. Fumaric
4. Formic
5. Acetic
6. Propionic
7. Isobutyric
8. Butyric
9. Isovaleric
10. Valeric
11. Media components
12. 4-methylvaleric
13. 3-(p-hydroxyphenyl) propionic
14. 3-phenylpropionic



Chromatogram courtesy of Guerrant, G.O., et al., *Journal of Clinical Microbiology*, **16** (2), 355 (1982).

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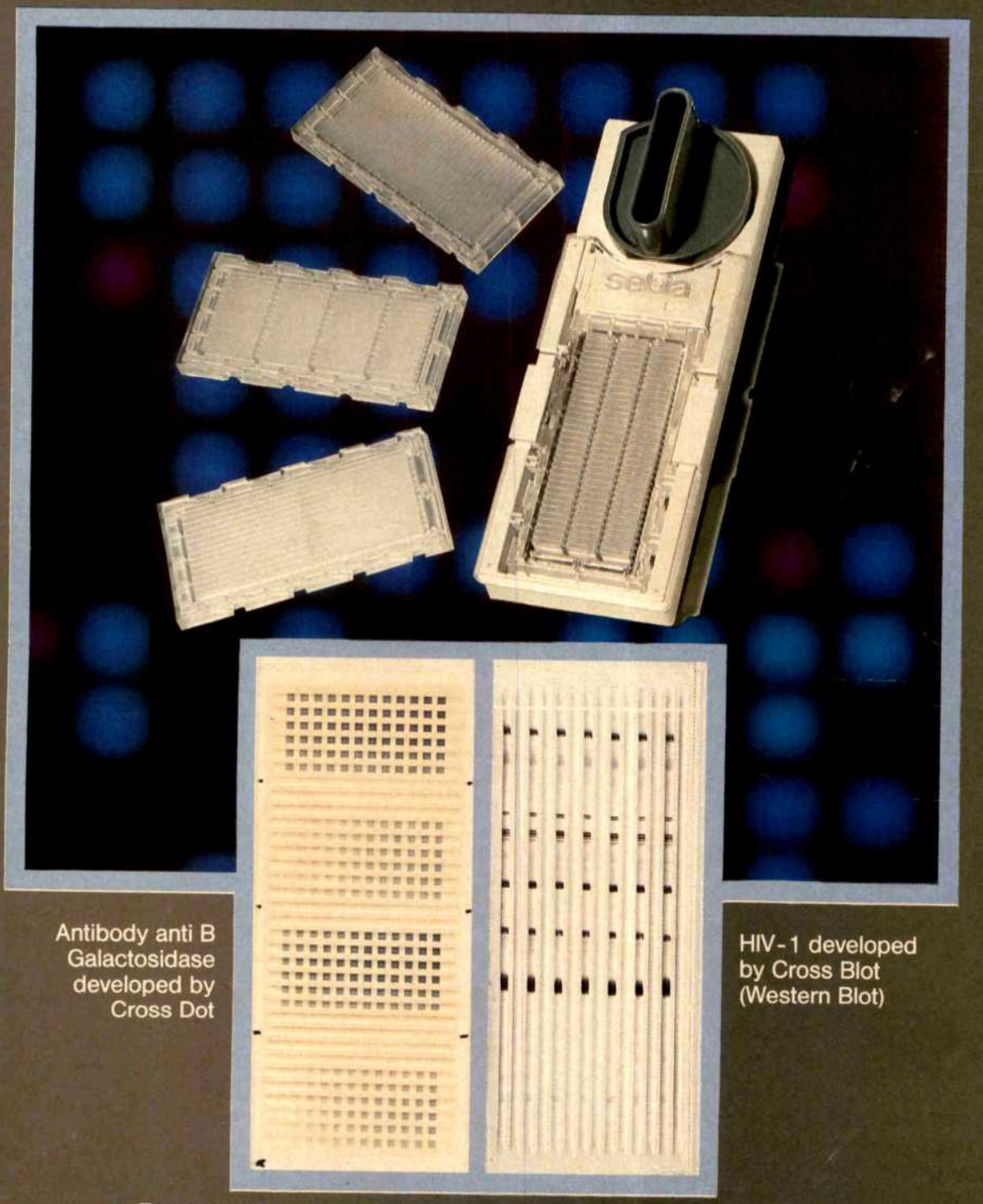

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Nature® ISSN 0028-0836
Registered as a newspaper at the British Post Office

Vol. 340 No. 6236 31 August 1989

NATURE VOL 340 nature 31 AUGUST 1989

AIDS now a tractable disease?

Hopes that drug therapy will improve the lot of those infected with HIV have brightened, but it is too soon to tell what the consequences will be for the general spread of the disease.

AIDS has come to stay. So much is vividly illustrated by the inexorable increase of the numbers of those infected with HIV (human immunodeficiency virus), especially in the United States. When more than one per cent of the sexually adult population carries the infecting virus, and when there is no prospect of a downturn, it seems plain that the disease may well become as important a source of mortality among those in middle life as, for example, tuberculosis was in the 1930s. But there is also some good news. The spread of AIDS infection by heterosexual sexual intercourse appears not to have been as rapid as some feared just two or three years ago; at least in the short run, that is something to be grateful for. And now, there is the prospect that drug therapy may at least abate the development of overt AIDS in infected people. That many individuals may be helped to live longer, perhaps even to avoid the development of overt AIDS, is naturally welcome, but it is even more important that drug therapy could serve as a firebreak in the spread of infection, eventually limiting the social damage AIDS will cause.

But optimism should be constrained. Compared with the magnitude of the social problems already occasioned in places such as the United States by the spread of AIDS, the new developments (see *Nature* **340**, 581; 24 August 1989) are but straws at which to clutch. The drug called AZT (otherwise the Wellcome drug known as 'Retrovir'), hitherto used in the palliation of overt AIDS, has been approved by the US Public Health Service as a prophylatic for use among those infected with the virus HIV. Experience so far seems to show that the onset of overt AIDS can be delayed, in about half those treated, for at least a couple of years (the duration of the trial). Then approval has also (and commendably quickly) been given for the preliminary testing in human beings of Genentech's clever synthetic protein, called immunoadhesin, made from molecules which combine the receptor molecule CD4 and part of the immunoglobin molecule IgG1: but the first step is to tell whether the drug can be safely used in people: clinical trials proper will begin only in 1990.

Only the first of these developments can influence the immediate course of the spread of AIDS, and it is too soon to tell what the effects will be. For one thing, the use of AZT will be limited by the scarcity of the drug, based as it is on a natural product derived from herring sperm. Another way of putting that is to say that not many people infected by HIV and excluded from health insurance schemes (often because of their infection) will be able to afford the annual cost of prophylaxis (in excess of $6,000) out of their own pockets. Moreover, the wider benefits of prophylaxis remain to be determined. When so little is known of the infectiveness of people carrying HIV at the various stages between first infection and the development of overt AIDS, it is too soon to know the extent to which general use of AZT among carriers of HIV will reduce the spread of infection. (The bizarre possibility that, by prolonging the period of normalcy of the infected, general use of AZT might increase the potential for spreading infection cannot be dismissed, but seems unlikely.) On general grounds, if eventually shown to be effective, Genentech's immunoadhesin (which would remove circulating virus from the bloodstream) promises to have a more direct influence on the infection rate, but only time will tell.

The practical question remains of how these potential benefits may be secured not merely for those infected, but for society at large (which has a vital interest in any impediment to the spread of infection). The difficulties should not be underestimated. The physical scarcity of AZT is something to be reckoned with, and at the beginning will almost certainly limit the use of the drug to those carriers of HIV whose T-cells are already depleted (which is what the US Public Health Service recommends), but that will not extinguish demand from infected people (and their physicians) in whom there are no pathological signs. The cost of this development, especially in the United States, will be considerable.

Even if most of those infected with HIV are covered by health insurance schemes, so that the whole cost does not come from their pockets, their treatment will not be free, but will be reflected in the insurance premiums that everybody pays. At present prices, the aggregate cost might exceed $1,000 million a year, by which yardstick Senator Edward Kennedy's proposal that there should be an extra $30 million in the US budget to cover the cost of treating the uninsured seems over-modest. Yet this is an area of public expenditure in which, the US budget deficit notwithstanding, even the possibility that the wider use of AZT will be a firebreak in the spread of infection should be seized with energy — and at whatever cost is necessary. The emergence of drug prophylaxis, however insubstantial it may be at present, is one of the few cheerful developments in the past five years. □

Conflicts of interest

The US Congress, now interested in conflicts of interest, should aim at a few prescriptive principles.

THE dispute that has come to light at the University of Pennsylvania (see page 668), and on whose rights and wrongs it is too soon to form an opinion, will at least provide further justification for the decision earlier this year of Congressman Ted Weiss, chairman of a congressional subcommittee to embark on a formal inquiry into conflicts of interest arising when academic research is supported both by industrial companies and by public funds. The inquiry is not merely proper but important. Yet nobody should jump to the conclusion that Weiss is about to uncover an area of academic life in which scandal is even more plentiful than the cases of outright fraud involving the misrepresentation of data that have been uncovered during this decade.

That commerce and academic research do not mix easily was dramatized by the first wave of excitement about the potential of biotechnology in the late 1970s, and has since been sharpened by the way in which governments have been urging on the academics who look to them for support that industrial interests should be more fully catered for in universities and research institutes. There are several pitfalls into which academics and their institutions may fall, ranging from the possibility that particular industrial companies may benefit unfairly from academic research projects (perhaps securing their advantage by appropriately genteel kickbacks) to the danger that the quality of research may be compromised (or its publication unduly delayed) by commercial interests.

To recite the sources of difficulty is not to argue that commercial interests have no place in academic laboratories. And since the social function of academic institutions is at least partly economic, it may be correctly argued that academic research institutions have a social duty to assist industrial companies in innovation and competitiveness. Striking a balance is naturally difficult, while it is plain that some governments (the British in the past decade, for example) go too far in their utilitarian demands.

These are issues for institutions to argue out with their sponsors. What interests the Weiss committee, properly, is whether commercial interactions affect the behaviour of individuals in research, with consequences that are either unseemly or downright inequitable. It will uncover in the months ahead, a lot of gossip, but its true goal should be to illuminate the general principles on which relationships between universities and industrial or commercial sponsors are regulated.

First, explicitness should be the general norm. If academics, singly or collectively (as a university department, for example), come to an arrangement with outside interests about their programmes of research, its basis should be fully disclosed. In one form or another, most academic institutions are in a position to claim that this is already done: there are usually formal limits on the amount of time academics can devote to consultancies and rules requiring that at least some official of the university should be kept informed. But rules of this kind are often inadequately policed, and are insufficient.

Disclosure should serve several purposes, of which one is that a researcher's immediate colleagues understand what he is about. It would, for example, be corrosive of trust in research that one member of a research group should not have told his colleagues in the same academic enterprise of an outside connection with some commercial company. Yet there are many universities in which the rules do not require even that degree of disclosure. And there is a general reticence about the sums of money that change hands. People may often satisfy the local regulations by disclosing that they have an outside arrangement, but may not say how much it is worth to them. The consequence is that the abatement of excess that colleagues might informally bring about does not apply. The general rule should be that disclosure should be full, and made public within institutions.

A second principle is that of how the financial rewards of outside commercial interests should be shared. As things are, most academic institutions leave the negotiation of outside arrangements to those concerned and let them keep the proceeds. If a university department takes on a research project for an outside company, it will usually recover the cost involved and an accompanying contribution to its overheads, but some of those carrying out the research may be rewarded separately. The matter of patent rights to important innovations is potentially even more contentious: while some universities, especially in the United States, have worked out arrangements by which they and successful inventors share the rewards of innovations, others have neglected the need to formalize these matters, to the general discontent. Nobody suggests that academics should not be paid, but there are merits in the simple rule that external earnings should be divided three ways, between the individual concerned, his department or research group (to support further research) and the whole institution. If the Weiss committee can win general acceptance of some such rule, it will do a public service.

A third principle concerns academic researchers who may become officers of independent commercial companies. In the past few years, people with a bright idea have sought to exploit it commercially by setting up a company, often appointing themselves as chairman, perhaps even chief executive. That is not merely unwise but also a serious threat to people's academic integrity. When companies run into serious trouble, their managers have a fiduciary duty to shareholders to drop everything else, doing what they can to save the commercial enterprise which is not compatible with academic life. The rule should be that academic researchers may be shareholders or non-executive directors, but never have management responsibility. Weiss should push for such an understanding. □

Voyager's final Solar System rendezvous

- High clouds and hurricanes on Neptune
- Triton marked by glaciers and ice volcanoes

Pasadena, California

As Voyager 2 rounded Neptune 5,000 km above the clouds and sailed within 25,000 km of the large moon Triton, it ended its spectacular 12-year mission with a flourish.

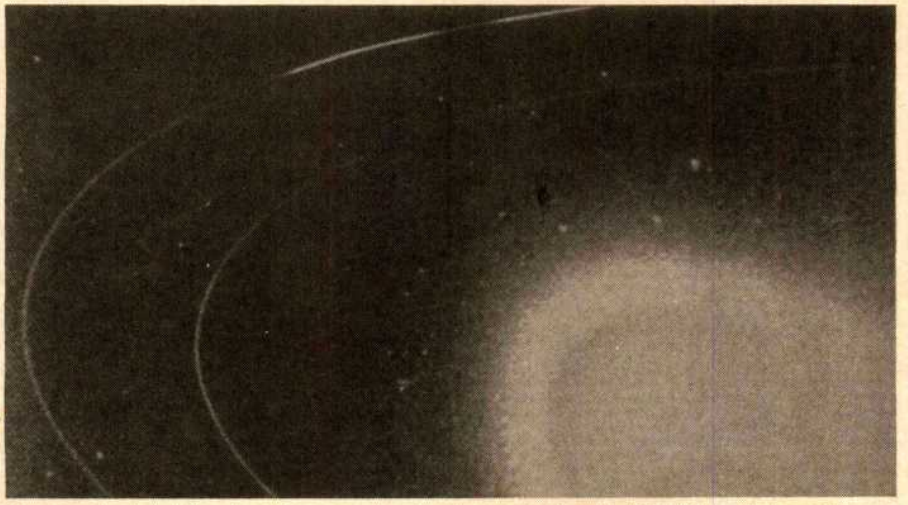
Neptune, the last of the giant gaseous planets in the Solar System, turned out to be a more visually appealing object than the generally featureless blue globe of Uranus. On Neptune, Voyager saw high cirrus clouds and a number of large visible features, some bright and some dark, that maintained their identity over the duration of close approach. And Voyager's close pass by Triton revealed a small world marked by recent and probably continuing activity: despite the intense cold, which makes water ice on Triton as unyielding as rock on Earth, Neptune's biggest moon is covered by flows, frozen lakes and 'icy volcanoes'.

Voyager 2, like Voyager 1, will now coast on towards interstellar space, and should remain in radio contact for another thirty years. It will send back measurements of the intensity of the solar wind, and may eventually cross the heliopause, where true interstellar space takes over from the region dominated by the influence of the Sun, but there is nothing more to take pictures of. At Voyager mission control, the Jet Propulsion Laboratory (JPL) in Pasadena, California, Vice-President Dan Quayle came to give the spacecraft a send-off, and as the stream of data from the encounter dwindles, the specialist teams will return to their home institutions to puzzle over their new discoveries. JPL is now turning its attention to the future (see page 669).

The atmospheric activity found on Neptune came as a surprise. Uranus, about the same size as Neptune and similarly shrouded in a dense methane atmosphere, presented a bland face to Voyager three and half years ago, and there was reason to think that Neptune, half as far again from the Sun and correspondingly colder, would be equally inactive.

But even a few months before the encounter, images from Voyager began to reveal large and conspicuous markings on Neptune. The most prominent became known as the Great Dark Spot, in recognition of its superficial similarity to Jupiter's Great Red Spot, and as Voyager neared its destination, a handful of other features appeared. A smaller dark spot moves around the planet close to the south pole, and as it grew in size on successive images, a bright spot appeared in its centre. Between the Great Dark Spot and the smaller one is the 'scooter', a small bright feature that moved around Neptune once every 16 hours, rapidly overtaking the dark spots, whose rotation period is closer to 17 hours.

Scattered across Neptune in a number of latitude bands are bright streaks that were instantly and fittingly described as cirrus clouds. Although a handful of cirrus features appear around the Great Dark Spot in fairly fixed positions, most of the high bright clouds changed from one rotation to another. This gave atmospheric dynamicists a problem: to estimate the circulation speed of the atmosphere, they need to see features that can be ▶



Surprises included five rings (two seen here) and a magnetic pole 50° off the rotation axis.

Navigating to Neptune

On 21 August, 4 days and 6 million km before closest approach, Voyager 2 made its final course adjustment, a sideways nudge in velocity of half a metre per second in an overall speed of 20 km per second. This delicate change, shifting Voyager's arrival point at Triton by 200 km, put it directly behind the moon with respect both to the Earth and the Sun so that two occultations could be observed.

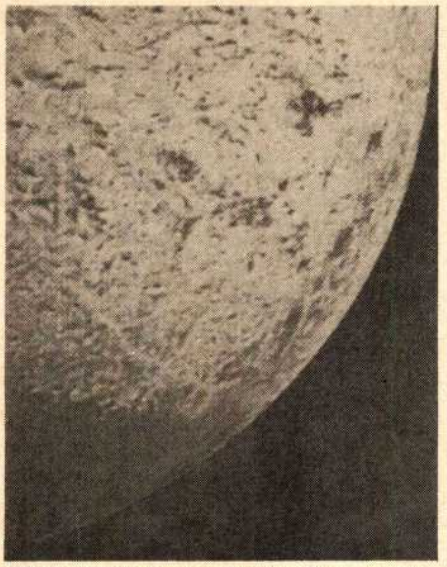
The apparently easy success of this fine-tuning of the trajectory conceals 12 years' worth of practised ingenuity on the part of the JPL scientists and technicians. Voyager's main thrusters, normally used for steering, cannot be fired during a planetary encounter because they heat up the radio receiver and, because of a long-standing malfunction, would cause a loss of contact with Earth for two or three days until the electronics cool down again.

During a planetary encounter, Voyager is steered instead with its attitude adjustment thrusters, which normally fire automatically every 20 minutes or so to keep the radio antenna pointed to Earth. Firing one of these sets Voyager spinning about the axis of the radio dish; when half a turn has been completed, another thruster can be fired to set the spacecraft rotating back the same way. Finally, the first thruster is fired again to stop rotation. The rocket firings are all in the same direction, perpendicular to the direction of motion; the net effect is to edge Voyager crablike across the sky, without loss of radio contact.

Only after this manoeuvre, when Voyager's passage through the neptunian system was calculated to an accuracy of 30 km, was the final set of instructions for the encounter prepared and transmitted. Some retargeting of the cameras was done to get close-up shots of two of the newly discovered moons, N1 and N2, and of segments of the rings.

On Wednesday afternoon, 23 August, Voyager's instructions for the encounter were transmitted. Because of the eight-hour interval between sending signals and receiving confirmation of their arrival, the command sequence was sent six times to ensure error-free receipt. Voyager's memory, state-of-the-art 12 years ago, can hold only 2,000 words of instructions: during the encounter, when the detectors must be turned constantly from one target to another, the meagre space is barely enough to hold two days' worth of instructions.

The finishing touch was delivered on Thursday morning, about 12 hours before closest approach. From final course determinations, an exact set of timings for the encounter was calculated and transmitted, telling Voyager when to execute the already installed series of commands. From this time until late Saturday afternoon, when it would be 2,500,000 km beyond Neptune, Voyager was on its own. □



Triton: there's no such thing as a 'dead world'.

unambiguously identified over several rotation periods. Around the Great Red Spot of Jupiter, for example, a multitude of recognizable smaller clouds pass by, permitting the rotation sense of the spot to be determined. But around Neptune's Great Dark Spot, the pattern of cirrus clouds is different on every image. Several days after the encounter, it was still impossible to decide if the Great Dark Spot is a cyclone or an anticyclone; as on Earth, the sense of rotation is different according to whether the spot is a low- or high-pressure region.

On some remarkable pictures, cirrus clouds are seen to cast shadows on the basal cloud layer; the height of the cirrus layer was estimated at about 50 km. The dark spots appear to be about halfway between the high and low cloud layers.

The high clouds are methane condensations; the lower clouds are thought to consist of hydrogen sulphide. Sunlight acting on methane in the upper atmosphere probably creates heavier hydrocarbons, which freeze and sink; lower in the atmosphere, where the temperature rises again, they evaporate and decompose back to methane, which rises in plumes to form the visible clouds. On Neptune, further from the Sun than Uranus, most of the energy available to drive atmospheric dynamics is internal heat, welling up from below, rather than solar energy impinging on the upper atmosphere.

Passing beyond Neptune, Voyager 2 saved some of the most startling images of its career for last. Triton, a cold and presumably icy place 2,700 km in diameter, was far from dead. At a quick glance, cratering on Triton seems not to be heavy, implying that the surface has been processed somehow in fairly recent times — the past 100 million years, for example. There had been suggestions that Triton would look 'new'; it has a uniquely odd orbit, circular but inclined to Neptune's axis, and retrograde. This makes it almost certainly a captured body, and suggests that in earlier times its orbit was probably not circular. As tidal forces circularized Triton's orbit, the strains on the moon's body would have heated it up, perhaps enough to melt water ice.

This indeed seems to have been the case. Parts of Triton's surface seem to have 'frozen lakes', resembling lunar maria. In places, the frozen lakes are stepped, suggesting a succession of meltings and re-freezings. But there is much more. Triton's inclined orbit, coupled with Neptune's, means that over a seasonal cycle of about a thousand years the Sun can be overhead anywhere from 50 degrees south to 50 degrees north. At the moment of Voyager's fly-by, the Sun was far to the south as seen from Triton, and near its south pole the surface markings were reminiscent of a martian summer, when the Sun is warm enough to evaporate the frozen carbon dioxide polar caps. On Triton, the ices are methane and nitrogen, but the process seems to be the same. Methane ice on Triton lends it a pinkish colour, darkly mottled by radiation damage from high-energy ionized particles which stream onto the the moon's surface and create darker hydrocarbons from the methane.

Elsewhere there is evidence of smaller flows that have filled in valleys and fissures. These are probably glaciers of methane and nitrogen which, like ice glaciers on Earth, can flow slowly over millions of years. Most bizarre of all are a few dark elongated streaks, tens of kilometres across. Initial suggestions that these might be wind-trails, formed of evaporating surface ices dispersed by the atmosphere, did not last long. Triton's atmosphere, inferred from the observation of a stellar occultation, has a pressure of no more than 10 millionths of Earth's, and is mostly nitrogen. There is too little of it to create a trail of sublimed ice.

The explanation settled on a few days after encounter was that the trails were from 'ice volcanoes'. Because nitrogen is just barely frozen at the surface temperature, an overburden of 20 or 30 metres is enough to cause nitrogen ice to liquefy. If fissures develop in the overlying ice, the liquid below will burst out, turn to gas which can shoot several kilometres above the surface, then condense and fall back. The thin surface layer so formed appears dark only in comparison with its surroundings, and may also entrap some of the sublimed dark ice particles in the vicinity.

A similar process may also account for yellow streaks seen on Io, one of Jupiter's moons, where underlying sulphur rather than nitrogen may rush up through surface cracks. Triton would then seem like a jigsaw of pieces from elsewhere in the Solar System, harbouring analogues of the martian polar caps, lunar maria and Io's vulcanism.

David Lindley

Rings, arcs and moons

Before Voyager got close to Neptune, many astronomers were anticipating confirmation of the existence of many 'ring arcs', or partial rings. But by early this week, only four or five rings had been found, and all were complete, encircling the entire planet. Where did the arcs go?

Observations from Earth of occultations of stars passing behind Neptune suggested partial rings: stars were seen to flicker in brightness as they approached Neptune's disk, but no diminution in brightness was seen as the star emerged at the other side.

Altogether there was evidence for six ring arcs, at various distances from the planet. The early signs from Voyager looked good. JPL scientists announced three weeks before the encounter (see *Nature* 340, 492; 1989) that they had detected faint reflected light from two partial rings.

But on Tuesday 22 August, new images revealed that the inner arc was in fact a complete ring, and that the other arc was longer than first thought. Much better pictures came down on Saturday, after closest approach, when Voyager could look back to see the ring system shining brightly in forward-scattered rather than reflected sunlight.

The full ring system revealed in these backlit images is complex. The two bright rings, 53,000 and 62,000 km in radius, are indeed both full rings, but the outer one has conspicuous clumps. Inside the inner bright ring is a faint and rather wide band, 42,000 km from Neptune and 2,000 km wide. Between the two bright rings is a fainter ring, but rather than a distinct ring this may be the edge of a tenuous, barely visible disk of material extending inwards to the inner bright ring, and possibly beyond.

The final assessment was that ground-based observers had indeed detected clumps in the outer ring — three of the observations gave about the right radius — and made the simple assumption that the ring was incomplete.

But one occultation measurement was both too far out and too strong to be consistent with any observed ring. Despite the astronomical odds, it seems that this occultation was caused by the then unknown moon 1989N2, a 200-km-wide body happening, at a distance of 500 million km, to pass in front of a background star.

The final haul of moons was six, varying from 42,000 km out and 50 km in diameter to 120,000 km out and 500 km across. As with the other giant planets, the rings and moons form a complex system, whose properties will take years to puzzle out. Neptune's clumpy outer ring demands explanation, but none is immediately to hand: none of the moons is in the right place or has the right mass to be clearly responsible, by its gravitational influence, for the non-uniformities observed. □

AUSTRALIAN BUDGET

Boost for Antarctic research

Sydney

In line with the prime minister's concern for the environment, the Australian government has greatly increased support for research in the Antarctic and on the greenhouse effect in its annual budget. One hundred and thirty-five projects, 10 per cent more than last year, will be allocated $59.8 million for the 1989–90 year. This is an increase of almost $16 million. About a quarter of the projects focus on research relating to the greenhouse effect, depletion of the ozone layer, research into the history of climate change and future climatic trends.

Much of the allocation will go toward a new Antarctic air-transport system to be tested over the summer months. Trials for an intercontinental Australia–Antarctica air link will involve two Royal Australian Air Force Hercules flights from Hobart in Tasmania to an ice runway at Casey station, one of three Australian permanent year-round research stations in the Antarctic. If successful, the trials may open the way for the introduction of a permanent Antarctic air-transport programme by 1990–91.

At present, travel to Antarctica from Tasmania can take up to six weeks by sea. According to Rex Moncur, acting director of the Antarctic Division of the federal Department of the Environment, an air link will not harm the environment. "Unlike the French, we are not using a rock platform, but an ice runway. The 2 per cent of the land mass that is rock is the major habitat of wildlife, whereas there is little wildlife on the ice. We will, however, conduct a two-stage environmental impact study just before the trial and a more detailed one before any full-scale air operation. An air link would mean that we could design research projects without having to consider whether we can get personnel or equipment in rapidly, and it will also encourage more senior people to do research in the Antarctic."

Funding has also been provided for the chartering of the marine research and supply ship, *Aurora Australis*. Since the sinking of the *Nella Dan* in 1987, Australia has been without a fully equipped marine research vessel. Two supply ships have been providing transport for passengers and cargo to and from the continent, but they have only limited research capabilities.

Unlike any other ship working the Antarctic, the *Australis* is designed to have no fuel tanks in its outer hull, making an oil-spill unlikely even in the event of an accident.

One of the first projects earmarked for the *Australis* will be to determine the amount of damage done by commercial trawling, particularly by Japanese boats. Other projects, both continuing and new, include investigations of the effect of increased ultraviolet radiation and carbon-dioxide levels on Antarctic and sub-Antarctic plants; research designed to limit the environmental impact of Australia's Antarctic research stations; analysis of ice core to determine the composition of the Earth's atmosphere in past ages; and, finally, studies of seasonal variations in atmospheric aerosol concentration and gas emissions from Antarctic waters.

Tania Ewing

HUMAN GENOME

Support for Japan's sequencers from MESC

Tokyo

The Science Council of Japan's Ministry of Education, Science and Culture (MESC) has decided to give a small boost to attempts to start a human genome project in Japan. But launch of a full-scale project is still a long way off.

In line with recommendations received from the Science Council earlier this month, the ministry is to provide ¥600 million (about $4 million) for a two-year preparatory study to be led by Kenichi Matsubara of Osaka University. The money, the first financial commitment to the human genome project by the ministry, will be released in September from 'emergency' funds, a source which shows that the ministry considers the project to be a matter of some urgency, says Matsubara.

The sum is comparable to that already being spent by the Science and Technology Agency to develop an automatic DNA-sequencing machine.

A decision on how the money will be used is not expected until next month. But Matsubara hopes that some of it will go to improve repositories and computer facilities, such as the DNA data bank at the National Institute of Genetics in Mishima.

The preparatory study, which will be carried out by about 20 researchers, including computer experts and biologists, will look at three key issues: training and recruiting of research staff, dissemination of results and ethical issues of genome sequencing.

Matsubara hopes to recruit many more researchers to the project over the two-year period. But he says that some researchers are concerned that the project may drain resources from other fields and he is trying to raise money from private industry and other sources. David Swinbanks

BIOTECHNOLOGY LICENSING

US opposition to milk hormone

Boston

In response to pressure from farm organizations and environmental groups, five of the largest supermarket chains in the United States agreed last week that their house brands of dairy products would not contain milk from cows injected with the genetically engineered hormone bovine somatotropin (BST).

The boycott by 2,500 supermarkets in the United States follows heated debate in Europe (see *Nature* 340, 415; 10 August 1989) and comes despite US Food and Drug Administration (FDA) approval for experimental use of the peptide hormone. When BST is injected into cows once or twice a month it can increase milk production by as much as 30 per cent. More than a hundred cattle herds have tested the hormone in the last four years.

FDA has yet to license BST fully for marketing and routine use but milk and meat from experimentally treated herds can be sold for human consumption. FDA representative Bonnie Aikman says that the data upon which that decision was reached will be published early next year in a scientific journal.

The announcement by the food companies came in response to a letter, challenging the companies to set out their positions, from the Foundation on Economic Trends, the group led by Jeremy Rifkin.

Paul Bernish, public affairs director at the Kroger Company, the largest US supermarket chain, affirms his company's belief in the role of the FDA to test new products to insure a safe food supply. But Bernish says "prudence" dictated his company's decision. "Until the government reaches a final decision", he says, "we simply would prefer not to have such products in our milk supply." Earlier this summer, the Vermont legislature called for a moratorium on the commercial use of bovine growth hormones pending a congressional investigation of its impact on farmers and consumers. Other major dairy states have introduced legislation to ban hormone-treated milk.

Rifkin's group and some 40 other farm and environmental organizations last week petitioned the FDA to stop all sale of BST-treated milk until final licensing is approved. They asked the FDA to reveal the location of test herds and to undertake a long-term study that will take into account the health of the nation's cattle, the possibility of long-term effects on humans and the economic impact on dairy farmers.

Aikman says that the FDA has yet to review the petition formally. But she stresses that the agency has no right to examine the economic effects of BST use. "We're here simply to rule on the issue of the product's safety." Seth Shulman

MISCONDUCT IN SCIENCE

Bitter dispute reaches NIH

Washington

NEXT month, an investigative committee from the National Institutes of Health (NIH) will be set up to look at one of the most tortuous biomedical controversies of the 1980s. Ostensibly, the committee will have the task of scrutinizing the scientific merits of a clinical trial of an antibiotic used to treat infection of the middle ear in children. But the dispute over the research, conducted at the Otitis Media Research Centre (OMRC) at the Children's Hospital of Pittsburgh, now involves allegations of misconduct and financial conflict of interest, and raises questions of the ownership of federally funded research and the proper role of scientific journals and university inquiries in the resolution of scientific disputes.

Charles Bluestone — under fire.

As in other quarrels that NIH have investigated, the two protagonists in the dispute — Professor Charles Bluestone, director of OMRC, and Professor Erdem Cantekin, former director of research for pediatric otolaryngology at the hospital — do not agree on even the simplest facts. They have not spoken to one another since 1986. At that time, Bluestone, who had asked Cantekin to join the department 11 years earlier, removed him from his post as research director. Cantekin now sits alone in an office at the end of a quiet corridor far removed from his former colleagues. Having faced three university investigations and a congressional hearing, Cantekin now leads the life of a full-time defendant.

The quarrel began with a scientific argument over the results of the trial. Cantekin claims that the research shows that the antibiotic under test, amoxicillin, is not effective in treating infection of the middle ear when compared with a placebo. He says that the results are flawed because the researchers relied too heavily on the physical observation of the ear with an otoscope, a technique which he believes is prone to observer bias.

Bluestone says that the antibiotic is effective and claims that Cantekin's analysis is flawed, in part because the endpoint of the trial was determined after the data were collected.

Who owns the data?

The dispute became public when, having failed to settle their differences, both researchers sent manuscripts to the *New England Journal of Medicine (NEJM)*. Faced with two views of essentially the same data, the editor Arnold Relman, decided that the journal was "in no position to make any judgement between them". Instead he wrote to the University of Pittsburgh for instructions as to which was the 'authorized' version.

Professor Eugene Myers, now dean of the University of Pittsburgh School of Medicine, co-signed a letter to Relman to express his "total and complete support for, and endorsement of" the Bluestone manuscript, which was the "only authorized manuscript". Cantekin's manuscript was rejected and Relman explained in a letter to Cantekin's lawyer that "the important question is . . . not whose interpretation is correct . . . but rather who has the right to publish the data first". Subsequently, *The Lancet* also rejected the manuscript, suggesting that it be published as a letter. But the *Journal of the American Medical Association (JAMA)* expressed "substantial interest" in the manuscript.

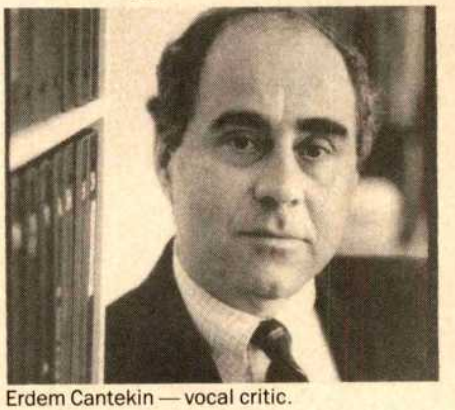
Answering questions at a congressional subcommittee hearing on conflict of interest in June, the editor of *JAMA*, George Lundberg, said that to ask an institution to say who are the "appropriate" authors "would undermine the trust relationship" between editors and authors. In the unlikely event that a journal should receive two manuscrips analysing differently the same data, it should review them both, he said. A journal should publish "the most truthful and important articles possible without being particularly concerned as to what the institutional politics might be". Cantekin's manuscript was reviewed by *JAMA*, but by the time the review process was complete, the university had begun misconduct proceedings against Cantekin. *JAMA* refused to publish the paper until these were over.

The misconduct hearings began when Cantekin accused Bluestone and Myers of obstructing his right to publish freely the results of publicly funded research, and of punishing him for his dissenting views. But these charges were dismissed and counter-charges of academic misconduct were made by Myers against Cantekin. Two university panels investigating the charges concluded that submitting the manuscript to the *NEJM* was unethical.

The investigations entered the final stages this month when Cantekin appealed to Wesley Posvar, the president of the university. But regardless of what he decides, the university may be unable to discipline Cantekin further. To strip him of tenure and remove him from the faculty would require the consent of the university senate. But the senate's committee on tenure and academic freedom has said that the School of Medicine's research integrity policy "puts the power of judgement in the hands of those most anxious to preserve a *status quo*". The committee in turn has been accused of bias by Myers, who says it has "never listened to the other side of the story" and is "always on the side of the underdog".

Conflict of interest?

Bluestone's behaviour has since become subject to further scrutiny after Cantekin claimed that he had failed to declare fully financial support from pharmaceutical companies, as required by NIH.



Erdem Cantekin — vocal critic.

No money was received for the disputed trial, but OMRC has received large sums since its foundation. The research carried out there is important to the pharmaceutical companies because infection of the middle ear is one of the most common childhood ailments. Doctors routinely prescribe antibiotics for it, at a cost in the United States of about $500 million a year.

The university says that in obtaining funds from pharmaceutical companies, Bluestone is "within the university guidelines" and that there is no concern about a conflict of interest. But a committee from NIH, which also looked into the allegations, recently sent to the director an interim report saying that it "found merit in the allegation that OMRC has not generally disclosed to NIH the extent of its industry-sponsored research". The report, which has not been made public, says that although a few studies supported by pharmaceutical companies were cited specifically, "no dollar amounts were given". But it also says that many institutions were found to interpret the instructions for grant applications improperly at that time.

The committee found that from 1983 to 1988, Bluestone received almost $300,000

in honoraria, about $260,000 of it from pharmaceutical companies. And since 1980, it says, OMRC has received almost $3.5 million from pharmaceutical companies. The committee says this "gives the appearance of a conflict of interest".

But the committee found no favourable bias toward drug companies in the research results, largely on the grounds that the "the number of positive and negative studies were approximately equal".

Bluestone says he did not disclose the amounts he received because he "misinterpreted" the NIH instructions, but he claims that full descriptions of the industry-funded studies were described in progress reports. Speaking at the June congressional hearing, James Wyngaarden, former director of NIH, said that before 1985, the instructions were not clear. When they were made more explicit, he said, it was "quite possible that he [Bluestone] just didn't notice the new requirements and neither did we".

Scientific dispute

The new NIH committee now has the difficult task of unravelling the scientific issues in the dispute. Wyngaarden said that "some of the rather unusual steps Cantekin has taken might be viewed somewhat differently if his scientific views are sustained". But an independent consultant who reviewed the manuscripts for the university hearing board said "it may be that replication of the trial is the only way to resolve the current dispute". Congressman Ted Weiss, chairman of the subcommittee that held the June hearing (see *Nature* **339**, 568; 1989), argues that the results of a subsequent study by OMRC also appear to show that amoxicillin is no more effective than a placebo. Bluestone denies this and says that the results were taken out of context. He also denies allegations made by Cantekin that he has intentionally delayed publication of the results. He claims that the manuscript is being submitted to *NEJM* this week. Cantekin asked to see a final report on the study prepared for NIH but his request was denied because the contents are patentable. The study was funded in part by the companies whose antibiotics were being studied.

Weiss is not satisfied with written answers he has received from Bluestone and plans to hold a hearing in the next few months at which Bluestone will testify for the first time. This case, he said "provides us with many examples of what should not be happening with NIH-funded studies". With its particular interest in how institutions handle misconduct cases, and in ensuring that whistleblowers are protected, the House of Representatives subcommittee on oversight and investigations, chaired by John Dingell, is also following the controversy closely.

Christine McGourty

NEPTUNE ENCOUNTER

JPL looks beyond Voyager

Pasadena

As the Voyager 2 spacecraft sped past Neptune last week and off into the furthermost reaches of space (see page 665), it marked the end of the first grand reconnaissance of the Solar System. It also highlighted a new era for the Jet Propulsion Laboratory, which manages the Voyager mission and many other unmanned space expeditions for NASA (the National Aeronautics and Space Administration).

After a stormy period of budget cuts, JPL is is basking in the warmth of President George Bush's support for space studies and is preparing itself for a new phase of exploration that will dictate its direction well into the next century.

Begun in the 1930s as an aeronautical laboratory for nearby California Institute of Technology (CalTech) and later serving as an Army research facility, JPL was turned over to NASA when the agency was created in 1958. Its assignment was to take the lead in the unmanned exploration of the Solar System. Besides the twin Voyagers, early missions included the Mariner series that explored the inner planets and the Viking journey to Mars. But while scientists are still reaping the benefits of these missions, the launch of the last Voyager in 1977 meant that JPL had to look to new venues for its bread and butter.

The time of the Voyager launch coincided with a period of waning federal support for the space programme that reached its nadir with budget and staff cuts in 1982. The crisis prompted a decision to accept funds from other government agencies — most notably the Department of Defense — and set off an uproar at both JPL and CalTech, which through a unique arrangement pays the salaries of the laboratory employees. But the move bolstered JPL's research base. And since 1983, the facility has enjoyed steady increases in support that have brought its annual budget to a little over $1,000 million and its staff to 5,200, of whom more than half are engineers and scientists.

The laboratory's strategy for the rest of the century and beyond focuses on three general areas — additional Solar System studies, investigation of the Earth itself and studies of other solar systems.

The first category includes some missions planned long ago but only now getting under way. Among them are the Galileo mission to Jupiter, which has an October launch date, and the Mars Observer, which is scheduled for 1992. Both are projects the laboratory had hoped to complete in the early 1980s. Future plans include the Cassini mission to Saturn, which will be the first JPL craft to employ 1980s technology. Also in the planning stages are efforts to build automated rovers that would land on planets and satellites and return surface samples to Earth. "We are now getting into a new phase of science — the '*in situ*' robotic exploration of the Solar System", says the laboratory's chief scientist, Moustafa Chahine.

For most of JPL's history, Earth had been ignored. Ten years ago, the laboratory formed an Earth and Space Sciences Division. More recently, JPL scientists began working on the Earth Observing System (EOS), a gigantic undertaking involving at least two space-based platforms, the first of which is due to be launched in 1996. All told, JPL scientists designed and developed 11 instruments — nearly a third of the total payload — that will fly aboard the two EOS platforms.

Each step in JPL's evolution has meant new developments in microelectronics, information systems, image processing and computer systems to help analyse the data gathered. One new system involves the hypercube, a parallel computer pioneered at CalTech. JPL scientists are working on a system, Mark 3fp, that interconnects up to 128 processing nodes that can each tackle the same or different tasks. The system will be able to combine data from different sources — microwaves, radio waves, infrared and visible light — into a multi-dimensional image that make it possible to see directly patterns in data that are normally hidden in piles of computer print-outs.

Despite such new projects, some critics say JPL is showing its age. Director Lew Allen disputes that vigorously, saying a crop of new scientists has moved in alongside the laboratory veterans. He is more worried that the long gaps between missions have left his crew rusty. Since one mistake can cost years of planning, he notes, "you clearly worry a great deal about whether your skills are up to snuff".

Robert Buderi

ANIMAL RIGHTS

Monkeys go home

Paris

A Lyons tribunal decided on Tuesday 22 August to order the return of 28 monkeys to the research laboratory from which they were stolen in May. The monkeys had been held at a primate centre in Strasbourg. Meanwhile, eight members of an anti-vivisection organization, Arche de Noé, are awaiting trial for the break-in.

During the night of 19 August, the Front de libération des animaux, broke into kennels in northern France, releasing 28 beagles belonging to a small company which carries out toxicity trials on drugs intended for humans. Peter Coles

COMPUTER NETWORKS

Japan ends its isolation

Tokyo

TOKYO University this month opened the first high-capacity computer link between Japan's national research institutes and scientific computer networks in the United States and other parts of the world. The link is expected to stimulate development of Japan's primitive computer networks and may also help to break down some of the bureaucratic barriers that exist between Japan's research institutes.

The new computer link, the Todai (Tokyo University) International Science Network, was established after Professor T. Nielson of the Department of Computer Information Science at the University of Hawaii approached Tokyo University's faculty of science late last year in search of a landing point in Japan for his Pan-Pacific Computer Network.

Unable to get government funding for the network, Akiyoshi Wada, dean of the faculty of science, with the help of Ken Sakamura of the university's department of information science, arranged for the giant computer manufacturer Fujitsu to support the project. Fujitsu has donated workstations for the network worth about ¥30 million ($210,000) and for the next 18 months the computer company will cover the 1-million-yen-a-month cost of leasing a high-capacity (64 kilobits per second) line between Tokyo University and Hawaii University.

The network will provide access to most of the scientific networks in the West such as HEPNET, used by high-energy physicists, SPAN, a network for astrophysicists, and LIFENET, used by life scientists. And Japanese scientists will be able to log onto computers in research centres in the United States and Europe as if the computer were "in their own room", according to Tsuneyoshi Kamae, chairman of the committee which established the network. Similarly, Western scientists will be able to log onto computers in Japan.

About ten national research institutes, including the High Energy Physics Laboratory (KEK), the Institute of Physical and Chemical Research (RIKEN), the Institute of Space and Astronautical Science (ISAS) and the Institute of Genetics, are expected to use the network, and these institutes and Tokyo University will pay for the network's running costs when Fujitsu's support runs out.

Surprisingly, the major running cost may not be the link with Hawaii but rather some of the domestic links in the network. The opening up of Japan's international telecommunications market to two new companies, including one backed by Cable and Wireless of the United Kingdom, has forced the former monopoly Kokusai Denshin Denwa (KDD) dramatically to reduce its rates. Even during the few months of planning the network, KDD reduced the price of the Tokyo-Hawaii link by more than 20 per cent.

But domestic telecommunication rates are still maintained at inflated levels by Nippon Telegraph and Telephone (NTT), despite the recent establishment of several small competing domestic telecommunication companies. For example, leasing an NTT line between Tokyo University and the Institute of Genetics in Mishima, a distance of about 120 km, costs more than half as much as the 10,000-km link to Hawaii.

Organizers of the new network hope to circumvent this problem by using the computer network of the National Center for Science Information System (NCSIS) which links Japan's universities and inter-university research institutes. NCSIS charges much lower rates than NTT but the capacity of its network (9,600 bits per second) is too limited. Tokyo University and KEK researchers, however, are requesting that it be upgraded to 48 kilobits per second.

Kamae says that computer networks in Japan lag far behind those of the United States and Europe. Computer companies and government ministries, he says, still maintain a "wartime attitude" about computers. They build huge mainframes "like the battleship *Yamato*" but not the small workstations that best suit computer networks. The workstations supplied by Fujitsu are made under licence from the US company Sun.

The Japanese government has built many huge "monuments" to science in the form of institutes and universities, Wada says, but "just as the navy built the battleship *Yamato* and forgot about logistics and communications", the government has failed to link Japan's research organizations into a coherent whole.

At the root of the problem is inter-ministry and inter-agency rivalry which prevents institutes and researchers belonging to different government organizations from interacting. But the new computer network seems likely to break down some of these barriers.

RIKEN is anomalous among the organizations joining the new network because it belongs to the Science and Technology Agency; the other institutes and the University of Tokyo are all affiliated to the Ministry of Education, Science and Culture (MESC). RIKEN will not be able to use the MESC-funded NCSIS network. Instead, a more expensive NTT line will be used. But by building up the number of users of the Todai network, Wada and Kamae hope to bring down the costs of subscription to a level that any institute or university in Japan can easily afford without recourse to special government support. **David Swinbanks**

OPTICAL FIBRE LINKS

Pacific cable breaks

Tokyo

THE new trans-Pacific optical-fibre cable (TPC-3) linking Japan and the United States has suffered a serious break in transmission only months after it began operations.

TPC-3 is the first optical-fibre link between the United States and Japan and began leased-line and telephone services in April. But on 18 July the line broke down and transmission was not restored until 22 August. The breakdown, which has not been reported in Japan, caused problems for workers at Tokyo University who are planning to use the cable for a computer link with the University of Hawaii (see above). Instead, the university has been using satellite transmission.

The break was traced to a point 4,500 km from Hawaii at a depth of about 5,000 metres. American Telephone and Telegraph (AT & T), which jointly operates the cable with Kokusai Denshin Denwa (KDD), replaced 60 km of cable and a repeater, according to Shigeru Minatani of KDD. The cause of the break is suspected to be a faulty repeater. David Swinbanks

NUCLEAR POWER

Alarm at plants

Paris

SERIOUS anomalies have been found this month in safety devices built into two French nuclear electricity generating plants. In both cases, temporary modifications to safety circuits were left in place after routine maintenance, rendering them essentially ineffective in the case of an accident. The first anomaly was detected earlier this month at the Dampierre plant.

Two plugs, fitted for tests on piping in the containment architecture, were not subsequently removed. Circuits designed to reduce the risk of an explosion in the event of a major accident were thus apparently compromised.

The most recent fault was discovered last week at the Gravelines reactor and was rated as a Class 3 incident (on a scale of six). Three safety valves, designed to prevent pressure build-up within the reactor in the case of an incident, were fitted with screws of the wrong type. It emerges that the screws had been in place since June 1988. The central nuclear installation safety service (SCSIN) has said that it is concerned by this apparent negligence. Meanwhile, Electricité de France, which operates the reactors, has ordered immediate checks at all its nuclear generator sites. Peter Coles

GREENHOUSE EFFECT

Looking at cirrus clouds

Munich

A UNIQUE high-altitude aeroplane will take off in West Germany on 17 September as part of a European airborne team that will investigate cirrus clouds and their effects on the climate. The clouds, which are made up of ice needles and play an important but little investigated role in global warming, will be studied in detail by the project, called the International Cirrus Experiment (ICE).

Stratolab will look at Cirrus clouds.

ICE, which is funded by four European governments — West Germany, Britain, France and Sweden — as well as the European Communities Commission, will involve simultaneous, parallel flights by up to five aeroplanes above, within and below the cirrus clouds 10 km above the North Sea. Equipment on the planes and on ships below will monitor the relationship between the ice crystals that make up the clouds and the clouds' ability to transmit heat or light. ICE is also expected to deliver important data on the physical properties of the crystals themselves, including their density and structure.

West German project leader Ehrhard Raschke of the University of Cologne says that ICE will complement the data about cirrus clouds obtained by the US-backed FIRE project (First International Radiation Experiment) of 1986. More aircraft will be used for ICE than for FIRE.

In order to maintain a stable climate, the Earth must return most of the Sun's radiation to space. Like other clouds, cirrus clouds allow sunlight to pass through, and when the same energy is re-radiated from the Earth's surface in the form of heat, they reflect it back to Earth. Because of the sparsity of measurements on cirrus clouds, they have remained something of an unknown quantity, but they are thought to play a potentially significant role in the greenhouse effect. The planes will fly up to three times a week for up to five weeks, although Raschke says he will be happy if six successful flights are made. The North Sea was chosen both because the researchers could obtain the necessary clearance from military and civilian authorities to fly and because it provides a homogeneous background against which to measure heat transfer. Project leaders also believe that the chances are good of finding cirrus there.

The high-flying plane, called Stratolab, is the first turboprop to reach altitudes of 53,000 feet (16 km). Originally called Egret, it is the product of a collaboration between the West German sailplane manufacturer Grob-Werke GmbH, the Texas-based electronics company E-Systems, and the private WIB-Berlin Space Institute. Stratolab was built to fly above 90 per cent of the atmosphere, into the middle of the protective stratosphere ozone layer there. It is the only European research plane that can fly so high.

Stratolab is effectively a sailplane equipped with an engine. At high altitudes, the plane can glide 40 to 45 metres for every metre of altitude lost. The propeller functions in the thin air at only 10 per cent efficiency, so gliding is a necessity. The plane could in theory carry two people, a pilot and a scientist, but in ICE there will be no passenger because the cabin is not pressurized. The pilot has to wear a specially fitted space suit. The Berlin institute, run by West German astronaut Reinhard Furrer, is currently working on equipment to measure stratospheric ozone and other trace gases using the plane. **Steven Dickman**

LITHUANIA

University reopens

London

THE 'Vytautas the Great' University of Kaunas is to reopen on 1 September, after a gap of nearly 40 years, in response to a public campaign in Lithuania.

The university was founded in 1922, at a time when Kaunas was the capital of the newly independent state of Lithuania. The university was closed down in the Second World War during the Nazi occupation, and briefly reopened under Soviet rule, but was then suppressed in 1950 as part of Stalin's clampdown in the Baltic republics. Although the university was replaced by a medical school and a polytechnic institute, the closure effectively deprived Kaunas of university-level education in the humanities and the natural sciences.

Vera Rich

ACID RAIN

China blamed for high pH

Tokyo

JAPAN is suffering from acid rain and China may be one of the sources, according to the results of a five-year nationwide survey released recently by the Environment Agency.

The first suspicions of acid-rain pollution in Japan emerged more than three years ago when researchers reported low pH levels in rain water and some damage to trees (see *Nature* **319**, 711; 1986). The agency survey at 29 locations around the country shows that acid rain (defined as rain water with a pH of less than 5.6) is falling throughout the country. But the average level of acidity at the 29 locations (pH of 4.4–5.5) is not as severe as in Europe and North America (average levels about pH 4.0). And the agency has not found any conclusive evidence of damage to trees, lakes or soil by acid rain. But it fears that damage may result even at these levels over the long term.

Japan implemented strict measures to combat air pollution in the early 1970s and as a result, emissions of sulphur and nitrogen oxides that cause acid rain have been kept down. But there is growing evidence that the continental mainland, in particular China, may be a source of airborne pollution, and with the rapid industrialization of China the problem is likely to get worse.

In 1986, the Environment Agency reported rising levels of a pesticide (benzene hexachloride) that is not used in Japan in a lake in the northern island of Hokkaido. And the agency suspects that the pesticide, which is used in China and Korea, may have been blown over from the continent (see *Nature* **320**, 478; 1986). Later the same year, the Shimane Prefecture Sanitation and Pollution Research Institute which faces the Japan Sea reported that strongly acidic rain (with pH as low as 3.1) frequently falls in the area. As there are no factories nearby, the continental mainland, in particular China, is again suspected. And the results of the present survey show that levels of sulphur and nitrogen oxides in acid rain on the Japan Sea coast increase in winter when prevailing winds blow from the continent.

China suffers from severe acid rain problems and during a recent visit to China by Japan's former prime minister Noboru Takeshita, Japan, at China's request, allocated ¥10,000 million ($70 million) in overseas development aid to build an environmental institute in Beijing that will have pollution monitoring stations around the country. The institute will carry out research and train state and municipal officials to deal with pollution problems. But as a result of the Tiananmen Square massacre, the project has been temporarily suspended. Hisakazu Kato of the Environment Agency, however, expects that the project will pick up again in "the very near future". **David Swinbanks**

Ethical dilemmas

SIR—David G. Potter (*Nature* **340**, 180; 1989) says "inescapable ethical dilemmas" exist in animal research and that "*Nature* can no more escape the moral responsibility when it publishes research involving animals than can be investigated".

It is interesting that Potter does not discuss the ethical dilemma of scientists being able to cure human disease, including mental disorders, by animal research, but being unable to do so because animal research has been outlawed. Is it not cruel for humans to suffer pain from diseases that could be curable by animal research? Nor does he discuss his moral responsibility to deny himself all medical care from previous animal research because he wishes to deny future generations the benefits of longer lifespans by future animal research, which Potter wishes to end.

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DNA fingerprinting

SIR—Do science and the law "differ in temperament"[1,2] to such an extent that evidence recognized as "true" by one would not recognized as such by the other? Surely any difference is rather that a greater degree of certainty is required when individual freedom, and perhaps in some cases life, is at stake, than when clues for further experimentation are being sought. If Eric Lander's advice that expert guidelines should be established for DNA fingerprinting[3] is taken, it is to be hoped that science and the law can achieve a consensus on what constitutes acceptable evidence.

There are two major uncertainties in any 'DNA fingerprinting' test — the reliability of the probe data with respect to the sample population and the reproducibility of data from a particular laboratory. The first problem could perhaps be addressed in a similar way to the identity parade, that is, suspects and 'controls' from a similar genetic background could be analysed in parallel. The problem of laboratory quality control is not unique to the forensic service and can be dealt with by having samples coded before analysis and by the inclusion of known samples (also coded) in each test. Statistical analysis of any laboratory's output would then be possible, enabling a continuous assessment to be made of the quality of its results.

On a practical note, variation of mobility of DNA fragments in gels is generally less of a problem in polyacrylamide gels, and the recent description of CA repeat-length polymorphisms[4,5] offers a new set of markers that could be used for fingerprinting[6]. Direct sequencing of amplified CA repeats would provide a very rigorous internal control for repeat-length polymorphisms.

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Misquoted

SIR—Commenting on the article "The case of the peripatetic fossil" (*Nature* **338**, 613; 1989), K. S. Jayaraman (*Nature* **338**, 694; 1989) said: "Gupta's colleagues at the Centre for Advanced Study in Geology, including its director, Dr A. K. Prasad, described Talent's allegations as 'a conspiracy to denigrate a top Indian scientist' ". This statement is misleading: Jayaraman did not consult us or other colleagues on this issue. As geoscientists working in the same institution, we are directly and deeply concerned with all aspects of the controversy and are alive to the need to find the truth.

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Raman's prize

SIR—The question of whether Raman deserved an undivided Nobel prize for his 1928 discovery of the light-scattering effect and whether Mandelstam's achievements in this area have been ignored by the Nobel committee still provokes some controversy[1]. But although it is true that some landmark discoveries made by Soviet scientists in this century have failed to receive adequate Nobel recognition, the selection of Raman over the claims of Mandelstam for the 1930 Nobel prize in physics is not a tainted one.

After attending the Sixth Congress of Russian Physicists, C. G. Darwin reported in *Nature* that Mandelstam and Landsberg "had independently discovered Raman's phenomenon, the scattering of light with changed frequency"[2]. Raman refuted this claim emphatically, by writing: "The Russian physicists, to whose observation on the effect in quartz Professor Darwin refers, made their first communication on the subject after the publication of the notes in *Nature* of 31 March and 21 April. Their paper appeared in print after sixteen other printed papers on the effects, by various authors, had appeared in recognised scientific journals[3]." This has not been repudiated by Raman's competitors.

According to the published census of Nobel nominees and nominators[4], for the 1929 Nobel prize, Raman received two nominations from N. Bohr and C. Fabry, while Mandelstam received none. In 1930, Raman received ten nominations, which included those of N. Bohr, E. Rutherford, C. T. R. Wilson and L. de Broglie (all previous Nobelists). In the same year, Mandelstam received two nominations and Landsberg one.

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Time-reversal

SIR—Would not one possible solution to the dilemma posed by Huw Price in "A point on the arrow of time" (*Nature* **340**, 181; 1989) be found in Guth's inflationary period? According to that, the Universe does begin in a highly disordered state at Time 0 but at a very early stage inflation sets in and establishes a condition of low entropy, at that point. We thereby have a disorder–disorder type of universe and Hawking has no need to find means of excluding it.

To speak of "temporality" or "atemporality" becomes a problem in semantics and not in physics, and time's "asymmetry" is no longer consequent on initial conditions but only on a subsequent inflation.

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SIR—Huw Price's philosophical discussion of time-reversal in a contracting Universe echoes the discussion put forward by physicist Thomas Gold at least 25 years ago, at a Cornell conference. A more 'popular' account later appeared in *The Runaway Universe*, by Paul Davies (Dent, 1978). Philosophers, it seems, still underestimate physicists as much as the latter underestimate the former. Or does time run more slowly in Australia, so that news of these developments has not yet reached Sydney?

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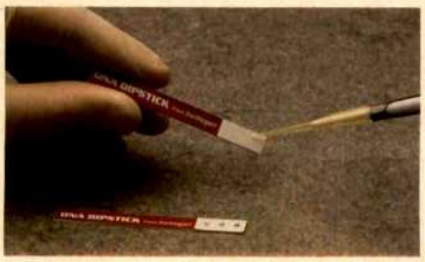

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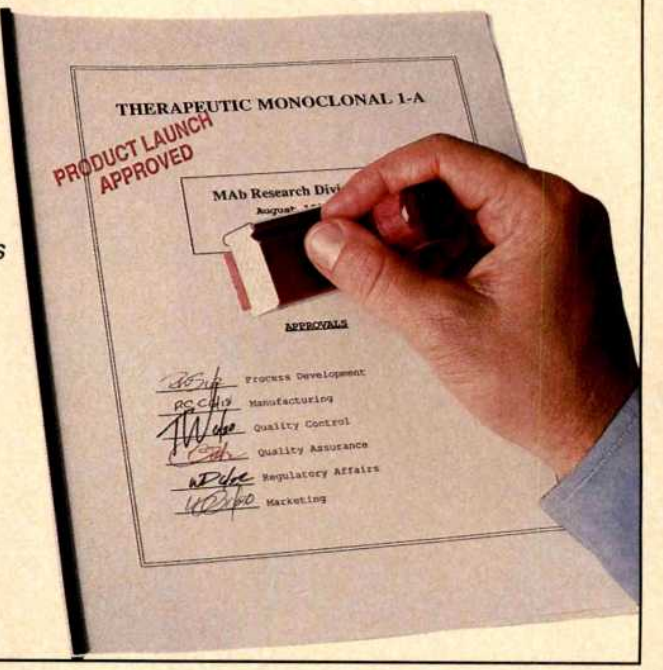

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Neptune's satellites predicted?

A theory believed to be successful in predicting the satellites of Uranus is not widely accepted. Much will hang on whether it has been successfully applied to Neptune.

Last week's encounter of Voyager 2 with Neptune will be, when the data have been analysed, a fateful occasion for Dr Andrew Prentice of Monash University in Melbourne. For the past several years, Prentice (a mathematician by trade) has had some success in predicting the properties of the outer planets and their satellites, most conspicuously at the encounter between Voyager 2 and Uranus in 1986. By definition, the encounter with Neptune will have been the last chance to test the accuracy of his prediction — there are no more planets left. But it is unlikely that the argument surrounding Prentice's work, which raises absorbing questions about the validity of predictive theories whose mechanisms are disputed, will be stilled as quickly.

Prentice himself makes no secret of the origin of his theory, which is based on the attempt by Laplace, in the eighteenth century, to relate the radii of the orbits of the planets to each other. Indeed, Laplace's theory was an important part of his great work *Exposition du Système du Monde* by which he is most widely remembered. For the period, the insight is quite remarkable. It is no shame to Laplace that he was unable to describe a mechanism whereby rings of gas and dust could condense into planets.

That is what Prentice reckons to have done, in the process reaching conclusions about the way in which the radii of the satellites of the multi-satellited planets beginning with Jupiter are related to each other. For the regular satellite systems such as those of Uranus, in which the satellites revolve in the same direction as the rotation of the planet, there is held to be a constant ratio between the radii of successive planets. This is the prediction that worked spectacularly well for Uranus. But Neptune is different in at least one respect: Triton, the largest of its satellites, travels in an orbit inclined to the ecliptic and to the plane of Neptune's equator, and in a direction opposite to that of the planet's own rotation. Few dispute Prentice's assumption that Triton was formed separately, but probably from the same material as Neptune (and, for that matter, Pluto). Part of his present interest is in the radii of the other pro-grade satellites of Neptune. Another is in the chemical composition (and thus the mean density) of objects such as Triton, as calculated from the supposed history of the condensing gas cloud.

The argument about Prentice's "modern laplacian theory" centres not on the predictions, but about the mechanisms it supposes to control condensation. The driving force both for the shedding of successive planetary rings and the formation of the separate satellite systems is supposed to be turbulence caused and then sustained by convection, presumably from the evolving primitive Sun. If there is such a mechanism, and if it can be sustained, there is no doubt that the consequences for a contracting solar nebula would be profound. In the outer regions of the cloud, for example, turbulent velocities would be supersonic, while turbulent stress would enormously augment the normal pressure of the gas. People's quarrel with Prentice hangs most simply on the circumstance that turbulence is a dissipative process whose effect would be to heat the gases involved in the turbulent motions (as might be expected for convection).

So what will people say if Prentice's predictions for the satellites of Neptune are shown to be as accurate as those for the satellites of Uranus in 1986? There will, of course, be two opinions. Prentice, his associates and his supporters will no doubt be confirmed in their opinion that they are on to something. Nobody would expect them to behave differently. But, equally, their critics will continue to insist that, in the absence of a plausible mechanism, the theory has no status.

Where will the truth lie? It is appropriate to recall that, in Laplace's time, strictly empirical theories were respectable.

Dulong and Petit, for example, contemporaries of Laplace, made sense of the specific heats of metals in a manner not made respectable until the arrival of the kinetic theory. Their rule, then a relationship between specific heat and atomic weight, should then, of course, have fallen into disrepute with the discovery that electrical conduction is mediated by electrons, only to be resurrected with the arrival of the quantum theory.

Then, at least, strictly empirical theories were both common and acceptable, as they still are in many fields — the various rules about species diversity with which ecologists are concerned are good examples.

So may it be that Prentice has arrived at an empirical rule for relating the properties of satellite systems to each other, and happens merely to have put forward an implausible mechanism that will eventually be refined? That, no doubt, is how the critics will be answered if the Neptune predictions are borne out by the observations of Voyager 2. And there will be some (but not Prentice, who does not acknowledge that his mechanism is faulty) who complain that too slavish a concern for mechanisms is the enemy of progress. That argument would have force if the issue were essentially practical, say the prediction of the superconducting properties of various ternary oxides (and, in the absence of an understanding of the mechanism of high-temperature superconductivity, empirical theories have enjoyed a new lease of life in that field during the past two years).

But there are two reasons why the licence does not apply to the prediction of planetary satellite systems, of which the simplest and more trivial is that there are only limited data to account for. With Voyager 2 now past Neptune, and with the prospect that it will be years or even decades before there is detailed information on other planetary systems, further prediction has no practical purpose. The situation is much like that in particle physics, where empirical arithmetical rules relating the masses of known particles to each other are forever popping up, but where the true objective can only be a surer understanding of their physical basis. In that connection, plausibility is essential.

The more telling reason why Prentice's critics will not fall silent is that there is already a great deal of successful planetary science entailing only plausible mechanisms. Indeed, it is one of the achievements of the past few decades that so much has been done to understand the way in which the Solar System formed from primaeval gas mixed with stellar debris. On that view, there was indeed a condensing nebula, the variations of chemical composition from one planet to another are intelligible in terms of the condensation temperatures of their constituents, but there must be many features of the Solar System that will be explained only historically, as consequences of past events still unknown. None of that is unrespectable, or a manifestation of orthodoxy conspiring to suppress the heterodox.

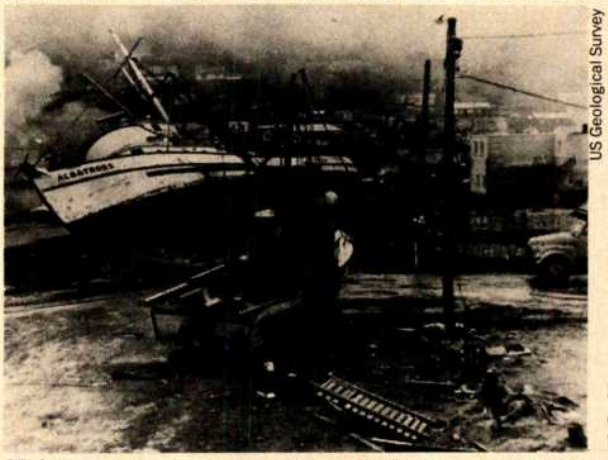
Even so, it will be interesting to see whether Prentice's predictions for Neptune are confirmed. **John Maddox**

EARTHQUAKES

Tsunami-wave seismology

Wayne Thatcher

TSUNAMIS (seismic sea waves) are among the most awesome and destructive effects of great sub-sea earthquakes. They result when fault slippage beneath the sea floor abruptly displaces the ocean bottom and generates a shallow-water gravity wave whose wavelength and speed depend on water depth at the epicentre and along the travel path. In open ocean basins, tsunami wavelengths are several hundred kilometres and their amplitudes are small and imperceptible. But near shore, the decreasing water depth and bottom friction cause wavelengths to decrease while amplitudes increase. The resulting waves, sometimes tens of metres high, have caused destruction and loss of life in coastal regions both near the earthquake source and thousands of kilometres distant (see figure). In new work (*J. geophys. Res.* **94**, 5627–5636; 1989) Kenji Satake shows that the information that can be derived from tsunami-wave arrival patterns can be as useful as conventional solid-earth seismology in determining the character of submarine earthquakes. Indeed, there are some respects in which tsunamis offer advantages over elastic-wave seismology.



US Geological Survey

30-foot tsunami waves raised by the 1964 Alaska earthquake caused havoc along the whole North American pacific coast.

Tsunami wave motion is most commonly recorded as changes of shoreline water level measured by tidal gauges, stilling wells set in the water and connected to the outer ocean by a tube or orifice. The recordings produced are in many ways analogous to seismograms and the use of tsunami waves in seismology has paralleled that of elastic waves.

Until about 1970, seismograms were used primarily to determine the arrival times of body and surface waves so as to locate earthquakes and to infer elastic modulii and wave speeds as functions of depth in the Earth along the route between an earthquake and its detector. First-motion directions and wave polarizations were used to constrain the orientations of earthquake fault planes and the direction of fault slippage. More recently, it has become possible to infer from the waveform information contained in seismograms detailed characteristics of earthquake sources and much better mappings of the three-dimensional elastic properties of the Earth's interior. Similarly until very recently, tsunami-wave seismologists were able to use only first-arrival time and initial-motion direction to estimate the spatial extent and direction of vertical displacement of the sea floor. But now it is becoming clear that tsunami recordings contain considerably more information about earthquake sources than had previously been appreciated: elastic-wave seismologists are watching new developments with interest.

The excitement is a result of the research by Satake, a Japanese seismologist whose work over the past 4 years has culminated in the publication of his new paper showing that tsunami waves carry information about the spatial distribution of earthquake fault slip comparable to that provided by conventional seismograms. Satake estimated the slip distributions in the 1968 Tokachi–Oki (M_w = 8.2) and 1983 Japan Sea (M_w = 7.8) earthquakes using arrays of 6–12 Japanese tidal gauges located within about 500 kilometres of these events. He shows that slippage over subregions of the earthquake fault plane as small as 30–50 km can be resolved by the regional tsunami waves and that the derived slip distributions resemble those independently obtained from seismic-wave analyses. The good spatial resolution of fault slip is due in part to the excellent distribution of regional tidal gauges in the Japanese islands, which have been densely instrumented since 1950. Elsewhere, and for earlier events in Japan, tsunami-wave inversions must rely largely on distant recordings which, because of their longer-period character, will have poorer spatial resolution of fault slip.

Satake's work has elicited widespread interest because of increasing awareness of the importance of slip heterogeneity and its influence on faulting processes. Heterogeneity creates slip-deficient zones with the occurrence of each great earthquake, and the behaviour of these zones from one cycle of seismic strain release to the next is important from several standpoints. If deficits generated in one cycle are made up by seismic slippage in the next strain release episode, then great earthquakes will differ significantly from cycle to cycle. If, on the other hand, there are even gross similarities from one event to the next, inter-earthquake aseismic slippage in the deficient zones will be required to match the long-term slip rates.

The relative importance of aseismic slippage, its spatial distribution and temporal variability all bear importantly on the mechanics of subduction and the nature of earthquake-related crustal deformation. Better definition of the regions of high seismic slip ('asperities') will contribute to elucidating their influence on earthquake mechanics (H. Kanamori *A. Rev. Earth planet. Sci.* **14**, 293–322; 1986). To the degree that earthquake slip patterns can be forecast, the expected strong ground-motion effects of recurrent events can also be estimated. Also, although it is thought that fault slippage in one large earthquake may affect the timing of the next comparable event (K. Shimazaki & T. Nakata *Geophys. Res. Lett.* **7**, 279–282; 1980), the influence of slip heterogeneity on earthquake recurrence is not yet well understood.

In this context, important questions about the tsunami-wave method include its typical resolution over the entire rupture plane and its advantages and shortcomings compared with seismic and geodetic techniques of slip estimation. For earthquake rupture zones lying entirely beneath the sea floor (as in the 1968 and 1983 events studied by Satake), tsunami data could elucidate the complete slip distribution, but movements on the deepest portions of the fault plane will be the least well resolved because they generate the smallest seafloor displacements. Even for these events, estimates of slippage at depth might be improved by including constraints from seismic or geodetic data. For subduction zone events whose deepest rupture segments lie below continents or island arcs, such information would be essential to obtain the complete distribution of earthquake slip.

A possible complicating factor in using the tsunami method is uncertainty about the degree to which sea-floor displacements reflect only the elastic deformation resulting directly from fault slip. It is widely recognized that some great tsunamis, such as those caused by the 1946 Aleutian earthquake (M = 7.4) and the 1896 earthquake at Sanriku, Japan (M = 7.9) were mostly the result of large-scale slumping of loosely consolidated sediments lying on the trench-arc slope. These movements are interesting in their own right and can be studied using tsunami-wave inversion methods, but as far as the estimation of seismic slip is concerned, they represent contaminating noise. Although these great earthquake-induced slumps are rare, it is not yet clear whether smaller events of similar type are more common and if so whether they contribute significantly to tsunami

waveforms generated by large earthquakes.

Despite these limitations, tsunami-wave methods have several advantages in the estimation of fault slip. Because of their low propagation velocities (around 0.2 km s^{-1}), tsunami waves generated from different segments of a great earthquake rupture will typically arrive at tidal gauge stations separated sufficiently to be readily distinguished. And because the period of tsunami waves (5–20 minutes) is much longer than the duration of rupture in even the largest earthquakes (several minutes), the final static fault slip distribution can be recovered from the data. Seismological methods of slip estimation are much cruder. Long-period bodywaves can provide qualitative mappings of regions of high slip (for example, see S.L. Beck & L.J. Ruff *J. geophys. Res.* **92**, 14123–14138; 1987), but because the seismograms are dominated by the effects of slip acceleration and deceleration in the few regions where strain release is highest, the distribution and magnitude of slippage elsewhere on the rupture is poorly determined.

Furthermore, seismic wave periods are not long enough to define the full static offsets in even the high-slip zones and, because lateral variations in the Earth's structure are imperfectly known, unavoidable uncertainties are introduced into waveforms corrected for source–receiver transmission effects. In contrast, tsunami propagation depends mostly on ocean depth, and supercomputers in use now are powerful enough to permit tsunami waveforms to be accurately corrected for the known effects of ocean bathymetry. Although land-based geodetic survey measurements of earthquake deformation are free of these shortcomings, they can constrain slippage only on the onshore or near-shore segments of the fault, typically less than a third of the entire rupture zone.

Given these advantages and tidal gauge records dating back to the mid-nineteenth century, the scope for new research is very broad. Sub-sea slip distributions can be estimated for many more circum-Pacific great earthquakes, some of which occurred before 1904, when modern damped seismographs were first deployed world wide. As a result, much more can be learned about the source characteristics of older earthquakes of considerable importance to seismology. For more recent events, further comparisons between tsunami-wave estimates of earthquake slip and those obtained by geodetic and seismic methods will better illuminate the advantages and shortcomings of each technique. □

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GALACTIC EVOLUTION

Starbursts, quasars and all that

Cedric Lacey

STARBURST galaxies, as the name implies, are the sites of abnormally rapid star formation. In the case of starburst nuclei, the most luminous examples, this activity occurs at the heart of galaxies that are otherwise normal and so have already converted most of their gas into stars[1]. On page 687 of this issue[2], Hernquist argues that the vast quantities of fresh gas needed to fuel a starburst can be driven into the galactic centre by the tidal interactions that accompany the merging of two galaxies. It may even be that starbursts, Seyfert galaxies and quasars are all part of a simple evolutionary sequence triggered by such mergers.

The activity of nuclear starbursts is confined to the central 1 kiloparsec of a galaxy, itself perhaps tens of kiloparsecs in diameter. The most dramatic examples are 10^{11}–10^{12} times as luminous as the Sun. Most of the radiation is emitted in the far-infrared — the optical and ultraviolet emission from the massive young stars being absorbed and re-radiated at longer wavelengths by a thick layer of dust that shrouds starbursts. The stars must form at rates exceeding 10–100 solar masses ($M_\odot$) a year to produce such luminosity even if, as the evidence[3] seems to indicate, only massive bright stars are formed. For comparison, the rate of formation of stars in our entire Galaxy is about 3 $M_\odot$ a year.

Starburst nuclei are also very rich in gas, containing perhaps 10^9–10^{10} $M_\odot$ of molecular hydrogen. Nevertheless, a starburst could consume this amount of gas in 10^8–10^9 years, a small fraction of the lifetime of a galaxy, the brightest starbursts being the shortest lived[4]. It is reasonable to infer that the gas is delivered to the galactic centre over a similar period, so that the influx is extremely large.

An interesting comparison can be made with the fuelling of active galactic nuclei — bright, compact sources of non-thermal continuum radiation, some of which is reprocessed by dust into thermal continuum radiation or by ionized gas into emission lines. The source is believed to be a massive black hole (10^8–10^9 $M_\odot$) that is accreting gas — at a rate of 10^{-3}–1 $M_\odot$ a year for Seyfert galaxies, which are as luminous as starburst nuclei, or up to a hundred times faster for quasars, which can be brighter still. Energy is released much more efficiently by the gravitational heating close to a black hole than by nuclear burning in a starburst, which is why active nuclei can be as luminous as starbursts, despite the smaller fuelling rates.

The source in an active nucleus is less than 1 parsec across, so that the gas must be fed into a much smaller volume than in starburst nuclei. It turns out that many of the brightest starbursts contain a Seyfert nucleus which emits a substantial fraction of the total energy, indicating that the gas-supply mechanisms in the two systems are somehow connected. Observations suggest that fuelling mechanisms are related to large-scale galactic dynamics: the brightest starburst galaxies virtually all show evidence of interacting tidally or merging with other galaxies[5]; and both Seyfert and starburst activity are often associated with the presence of a stellar bar[1,6] (an oval-shaped enhancement in the surface density, in the centre of the disk, which rotates like a rigid body).

This is the starting point for Hernquist[2], who has modelled the effects of a merger between a parent galaxy like the Milky Way, containing stars and gas in a disk, and a smaller, satellite galaxy that contains stars only. Initially, the satellite is orbiting at the edge of the disk, but it spirals in as it loses energy and angular momentum by dynamical friction against the parent's stars and gas. The stars end up gaining energy and angular momentum, but the gas ends up losing both, the reason for the difference being the dissipative nature of the gas. The gravitational field of the satellite perturbs the gas orbits from their previously circular form, causing gas streams to intersect and shock, building up regions of high density. Under the influence of this gravitational field, some of the high-density gas collects into a massive, gravitationally bound cloud, which sinks to the galactic centre by dynamical friction against the stars. Meanwhile, the satellite galaxy is tidally disrupted. The net result is that about a third of the gas initially in the disk — around 2×10^9 $M_\odot$ — is deposited into a central region less than 400 parsecs in radius. The entire merger takes about 10^9 years, but the gas cloud, once formed, sinks to the centre in only 10^8 years.

The simulations thus indicate a plausible mechanism for rapidly injecting large quantities of gas into the central regions of galaxies, but they do not answer the question of what happens to the gas once it gets there: they do not include any treatment of star formation, and the spatial resolution is limited to around 400 parsecs. It is certainly plausible that the large gas density built up at the galactic centre should result in vigorous star formation, owing to the reduced timescales for cloud–cloud collisions and gravitational instability and fragmentation[7], but there is no reliable quantitative theory for this. Once stars are being formed, the subsequent supernova explosions of dying massive stars will heat the gas by driving shock waves into it. Neglect of this effect is the

most serious defficiency of the simulations, because it could greatly inhibit the processes tending to concentrate the gas into the centre.

Because of their limited resolution, the simulations provide information neither on how a central black hole might be formed, nor on how an already existing black hole might be supplied with gas. There have been various proposals for the latter process. Norman and Scoville[8] have suggested that gas drag or dynamical friction causes massive gas clouds to spiral down to a 10-parsec radius, where the gas forms a dense star cluster. Gas shed by stars in this cluster as they die is assumed to form and fuel the black hole. An alternative picture[9] is that a bar instability develops in the central gas disk once it becomes sufficiently massive. The bar loses angular momentum by gravitational torques, again carrying the gas down to a small volume, after which turbulent viscosity sets in, driving accretion onto the black hole.

As the rate of star formation decays with the consumption or dispersal of the gas and dust surrounding the central source, the external appearance of the central region will change: at first, the light from young stars, re-radiated by dust, will be seen; then there will be thermal emission from dust and non-thermal continuum from the active nucleus, both in the infrared; finally, with dust removed, optical emission from the active nucleus could become apparent if there is still sufficient accretion onto the black hole. Thus, one may have the evolutionary sequence of starburst → Seyfert → classical quasar. If all that is needed to trigger this sequence in a spiral galaxy is for it to have a moderately sized companion, many spiral galaxies may have gone through such a phase at some time. □

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MARROW TRANSPLANTATION

Can cord blood be used?

David C. Linch and Leslie Brent

TRANSPLANTATION of HLA-matched bone marrow is currently the treatment of choice for selected patients with aplastic anaemia, leukaemia and inherited disorders of the bone marrow. The availability of HLA-identical sibling donors limits this option to roughly a quarter of otherwise suitable patients, so that panels of HLA-typed individuals willing to donate bone marrow have been set up in many countries. The panels need to be very large — a pool of 250,000 donors is estimated to have a 59 per cent chance of providing a compatible donor for any one patient, and with increasing pool size there is a disproportionally smaller increase in the number of successful matches[1]. In a recent report, Broxmeyer *et al.*[2] make the intriguing suggestion that the neonatal blood retained in the placenta contains sufficient blood stem cells to serve as a transplant inoculum. Neonatal blood could be collected, largely from the umbilical cord, and cryopreserved to build up a large bank, thus overcoming some of the difficulties associated with volunteer panels. The logistical problems associated with the creation and maintenance of such a bank are, however, quite formidable.

It was shown a quarter of a century ago that the blood of fetal mice contains large numbers of haemopoietic stem cells detectable in spleen colony-forming assays[3]. Likewise, second-trimester human fetal blood has a high incidence of haemopoietic progenitor cells estimated by *in vitro* colony-forming assays[4]; although the frequency is known to decline during the course of gestation, umbilical cord blood contains a higher number of progenitor cells per unit volume than adult blood, and it is roughly equivalent, in this respect, to adult bone marrow. This is amply confirmed by Broxmeyer *et al.* in over 100 cord blood samples. It is an article of faith that when there are large numbers of haemopoietic stem cells there will be large numbers of transplantable stem cells, but this is a reasonable assumption.

The novel data reported by Broxmeyer *et al.* concern the volume of cord blood that may be extracted: over 50 ml on average, but nearly 200 ml using an "optimized" method involving removal of blood from the maternal end of the transected cord whilst the placenta is still *in utero*, to which is sometimes added blood obtained by needle aspiration of engorged vessels on the fetal surface after expulsion of the placenta. This figure is surprisingly high — previous estimates[5] of fetal blood volume in the placental vessels immediately after birth have been of the order of 75–125 ml.

A 100-ml sample of cord blood can be expected to contain about 2×10^6 myeloid (GM-CFC) progenitor cells and 1×10^6 erythroid progenitor cells, which should be sufficient for reconstitution after allogeneic transplantation[6] provided that the stem-cell/progenitor-cell ratio is not appreciably less than in adult bone marrow. Only clinical studies can prove this point.

There are, however, two possible difficulties with the strategy proposed by Broxmeyer *et al.*, both relating to the possible development of graft-versus-host (GVH) disease. First, it is common practice when transplanting matched unrelated bone marrow to deplete it of mature T lymphocytes in order to minimize the GVH response; we are, however, told by Broxmeyer *et al.* that any fractionation of neonatal blood results in unacceptable losses of progenitor cells. This could be surmountable.

More worrying is the second possibility — that neonatal blood is contaminated by maternal cells. Some degree of leakage from fetus/neonate to the mother occurs in up to 50 per cent of births, usually during parturition, and occasionally clinically significant leakages occur from mother to fetus[6]. The incidence of relatively minor leakages from the maternal to the fetal circulation during parturition is not known, but it could be high. The magnitude of maternal blood contamination could be increased by the vigorous venesection from placental vessels before and after delivery of the placenta that seems to be required for the collection of optimal amounts of blood. Although adult blood possesses relatively few stem cells, the transfer of mature T lymphocytes incompletely matched with the histocompatibility antigens of the recipient could contribute unacceptably to GVH disease. Neonatal blood is virtually as immunocompetent as adult blood so far as the generation of cytotoxic T lymphocytes is concerned[7] and therefore has the potential for inducing GVH disease.

Finally, Broxmeyer *et al.* suggest that cryopreserved cord blood cells could be used for autologous bone marrow transplantation. The prospect of selected individuals (presumably the babies of the wealthy) having their cord blood stored as an insurance against the ravages of leukaemia or nuclear accident in adult life is more than a little disturbing. □

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HIGH-ENERGY PHYSICS

Beaming in on the Z^0 particle

David J. Miller

WITH four particle accelerators now available with sufficient energy to produce the Z^0, one of the most interesting particles in fundamental physics, many exciting results can be expected. Preliminary data from two of these accelerators, the Fermilab 'Tevatron' proton–antiproton collider[1] and the Stanford Linear Collider[2] (using electrons and positrons), are published in the 14 August *Physical Review Letters*. These data have been used to measure the Z^0 mass and the width of its excitation curve (how the production rate varies as a function of the particle's mass; this is linked to its lifetime by the uncertainty principle). These are among the most fundamental of the many parameters in the standard model of elementary forces.

The Z^0 mass is directly linked to the way in which the weak force 'mixes' with the electromagnetic force in electroweak theory. Its width is determined by the strength of its couplings to all possible pairs of particles into which it can decay: these include leptons (electrons, $e^{\pm}$, muons, $\mu^{\pm}$, or taus, $\tau^{\pm}$, all of which are relatively easy to recognize) or quark–antiquark pairs which produce jets of strongly interacting particles (protons, pions and so on). The new data are not yet sufficiently precise to make definitive tests of the standard model, but they show how rapidly the measurements are improving.

Because of the large width and short lifetime of the Z^0, its presence can be detected only by measuring its decay products. At Fermilab, researchers have recorded 132 muonic events and 64 electronic ones. (Decays to strongly interacting particles are masked by competing events in which these are produced directly in the proton–antiproton collisions.) The mass of each Z^0 has to be reconstructed from the directions and energies of the two muons of electrons from the decay. From the distribution of these masses the authors find the average value of the Z^0 mass to be 90.9 ± 0.3 GeV, and the resonance width, 3.8 ± 0.8 (statistical) ± 1.0 (systematic) GeV.

The Stanford Mark II detector can identify strongly interacting particles from Z^0 decays as well as leptons, and has detected 94 pairs of these, together with 12 muon and tau pairs[2]. The Stanford researchers scan the collision energy through the resonance zone, and count the rate of Z^0 production. These data have to be normalized, achieved by counting the rate of small-angle electron–positron deflections which occur electromagnetically. The resulting Z^0 mass is 91.11 ± 0.23 GeV, with a width of $1.61^{+0.6}_{-0.43}$ GeV.

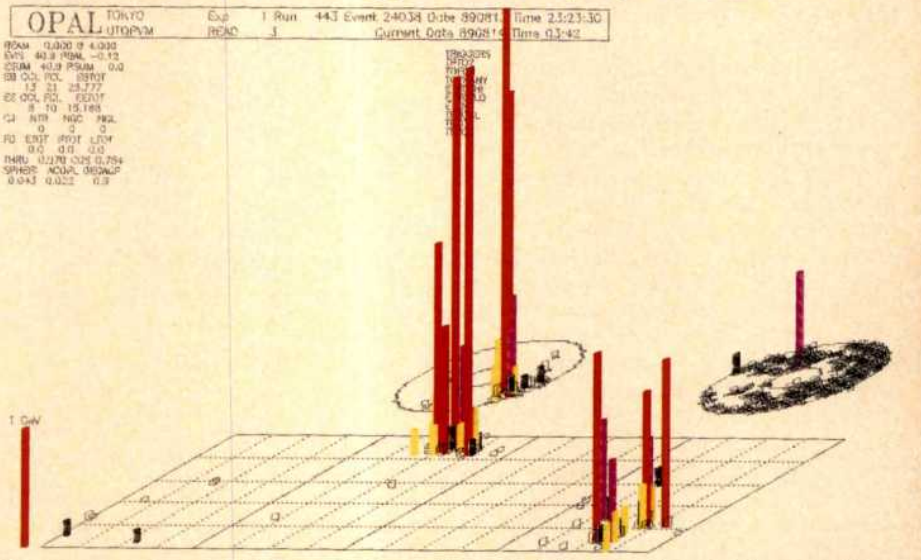
These results are much more precise than previous values, obtained at the CERN SPS proton–antiproton collider. The published Stanford width is particularly interesting because it is more than one standard deviation less than predicted by the standard model of electroweak interactions. Since the paper was submitted for publication, a further 140 Z^0s have been detected. Preliminary analysis of these, reported at a recent meeting*, suggests that the resonance is in fact a little wider, but it is still more than one standard deviation less than the predicted value.

If borne out by further measurements, the reduced width would deliver the first blow in 20 years to the standard model. One modification to the model would be to allow for additional, heavier Z^0s that share the weak neutral coupling with the standard Z^0; these would reduce the width of the standard particle's resonance. Instead of making the Z^0 width the variable in fitting the measured resonance curve, the Stanford group also uses an alternative approach in which the standard-model value of the width is assumed and the number of Z^0 decays to neutrino–antineutrino pairs is determined. These decays are invisible, but still contribute to the shape of the resonance. The Stanford group finds the number of light neutrino types to be 3.8 ± 1.4, on the assumption that each kind has the same coupling as the well known electron and muon neutrinos.

The number of neutrino types is directly linked to the thermodynamics of nucleosynthesis during the Big Bang, determining the primaeval abundance of helium in the Universe. We believe there are at least three types of neutrinos (the partners to electrons, muons and taus). The new result would allow two more types — not yet sufficiently precise to constrain the helium abundance to within astrophysical

* Lepton–photon symposium, Stanford, 5–11 August 1989.

Early Z^0s encourage CERN physicists



FOLLOWING a one-week trial run, the Large Electron–Positron collider (LEP) at CERN is the fourth accelerator to produce Z^0 bosons, the first being the Super Proton Synchtron at CERN, with Stanford and Fermilab succeeding recently. Z^0s have been detected in all four of LEP's underground experiments, although inevitable teething problems occurred on the accelerator, first commissioned little over a month ago.

The figure shows a 'lego' plot (courtesy of Tokyo University) of one of the first Z^0s to be detected at LEP, by the OPAL collaboration. The graph, which earned its title from the original method of photographing stacked Lego bricks, depicts the energy flow recorded in each of the lead glass blocks in a 'calorimeter' surrounding the interaction zone at OPAL. The calorimeter responds to electromagnetic showers caused by electrons or γ-rays. In this event, most of the signal probably came from γ-rays due to the decays of strongly interacting particles in the two narrow jets from the decay of Z^0 to a quark–antiquark pair.

In the plot, the cylindrical walls of the barrel-shaped calorimeter are rolled out flat with the end caps shown separately; the height of each bar is proportional to the energy deposited in a glass block.

The preliminary experimental run temporarily interrupted the commissioning programme for LEP, which has now been resumed. Among difficulties encountered during the run was an unexpected tendency for the beams to spread out vertically as a function of momentum (dispersion) even though there are no vertical bends in the machine. Nevertheless, physicists at CERN are greatly encouraged to have detected so many Z^0s — 50 in all — at such an early stage in its programme. □

limits — but it is getting closer.

If the width does turn out to be compatible with the standard model, the race will be on between Stanford and the new Large Electron–Positron collider (LEP; see box) at CERN to determine the Z^0 mass with a precision better than 50 MeV. As described by John Ellis in his recent review article[3], with this kind of resolution, it should be possible to constrain tightly predictions of the mass of the top quark, the second quark — as yet unobserved — in the heaviest known generation. What is more, if the top quark can be observed at Fermilab, which generates collisions of the highest energy currently available, its mass together with that of the Z^0 could be used to predict the mass of the Higgs boson, first postulated nearly 30 years ago to explain the masses of all particles in ordinary matter[4]. □

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G PROTEINS

Gasp: not just another oncogene

Frank McCormick

THE pathways that lead from the activation of cell-surface receptors to cell division remain obscure. Many of the stepping stones along these pathways are proteins encoded by proto-oncogenes: mutations in these genes result in proteins that cause uncontrolled growth. So far, we have not been able to connect the biochemical properties of these proteins and cannot fully describe how any one of them works. On page 692 of this issue[1], however, Henry Bourne and co-workers describe a putative oncogene that lies on a well trodden path connecting a surface receptor to a familiar second messenger with well documented physiological effects.

The product of this gene is a mutant version of $G_{\alpha s}$, the GTP-binding subunit of the G protein G_s which normally transmits signals from the β-adrenergic receptor to adenylyl cyclase, thus causing synthesis and accumulation of the second messenger, cyclic AMP. The possibility that $G_{\alpha s}$ could be an oncoprotein had been anticipated by Bourne in a previous News and Views article[2]. Commenting on a report that a subset of pituitary tumours contains elevated cAMP levels and increased rates of growth-hormone secretion, Bourne proposed that these effects were due to constitutive activation of $G_{\alpha s}$. Furthermore, cAMP was known to promote growth of pituitary cells, hence the suggestion that activation of $G_{\alpha s}$ could also lead to uncontrolled proliferation.

Bourne and co-workers now report[1] that in pituitary tumours with elevated cAMP levels and growth-hormone production, $G_{\alpha s}$ is activated by somatic point mutations. These mutations are in one of two amino acids: Arg 201, known to be a site of ADP-ribosylation by cholera toxin, and Gln 227, which is equivalent to Gln 61 of the *ras*-gene-encoded p21 proteins (Fig. 1). Both mutations (like ADP-ribosylation by cholera toxin) destroy the intrinsic GTPase activity of $G_{\alpha s}$, so that the protein remains in its active, GTP-bound state constitutively (Fig. 2). The activated form of $G_{\alpha s}$ is referred to as Gsp (pronounced gasp).

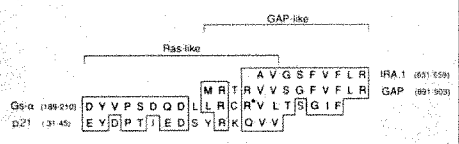

FIG. 1 Comparison of GTP-binding protein sequences using single-letter codes.

These results are reminiscent of the events that convert *ras* genes to oncogenes, and, as Bourne and colleagues point out, there are interesting comparisons between the two systems. By contrast with $G_{\alpha s}$, p21 proteins have very little intrinsic GTPase activity. They depend on a second protein, GTPase activating protein (GAP), to convert bound GTP to GDP. Mutations of p21 commonly occurring in human tumours (amino acids 12, 13, 61) prevent GAP from performing this function. $G_{\alpha s}$, on the other hand, has 'built-in' GAP activity, which confers relatively high GTPase activity. This activity is likely to come (directly or indirectly) from the Arg 201 region of $G_{\alpha s}$.

The crystal structure of the *ras*-encoded p21 in its GTP-bound state has recently been determined by Pai and co-workers[3] at the Max Planck Institut in Heidelberg. The regions of p21 that surround the bound GTP (including the Gln 61 region) have highly conserved counterparts in $G_{\alpha s}$ and all other known G proteins. It is therefore likely that all their GTP-binding sites will have very similar structures. However, *ras* p21 does not have an obvious counterpart to Arg 201 of $G_{\alpha s}$. What, then, is the role of this amino acid in $G_{\alpha s}$ GTPase activity? Inspection of the sequence of $G_{\alpha s}$ around position 201 reveals two interesting homologies, one with p21, the other with GAP, each suggesting different roles for arginine 201.

The region of p21 that resembles amino acids 189–203 of $G_{\alpha s}$ is the well-known effector-binding site[4,5]. Certain mutations in this region (amino acids 32–40) knock out biological activity of p21 without affecting nucleotide binding or membrane localization. It is therefore considered a likely site for binding of an essential target molecule (hence the name). Most mutations that destroy biological activity also destroy interaction with GAP, indicating that GAP is itself an effector of p21 action[6]. Another interesting feature of this region, revealed by the crystal structure determined by Pai and colleagues, is that Thr 35 coordinates the magnesium ion in the binding site and hydrogen bonds to the γ-phosphate of GTP. This region of p21 is likely to undergo a conformational change when GTP is hydrolysed to GDP, and may thus allow effector molecules to distinguish between GTP- and GDP-bound forms of p21. GAP, for example, binds to p21 only in the GTP-bound form[6]. In $G_{\alpha s}$ the equivalent residue is Ser 193. If this analogy is correct, mutations at $G_{\alpha s}$ residues equivalent to those defining the *ras* effector-binding region (such as Pro 192, Asp 196) might interfere with adenylyl cyclase activation. Also, if the structures of p21 and $G_{\alpha s}$ are similar in this region, then it is unlikely that Arg 201 could form part of the GTPase site of $G_{\alpha s}$ as it would be a considerable distance away from the bound nucleotide. We would then predict that mutations at this site, or ADP-ribosylation by cholera toxin, would produce conformational changes in the

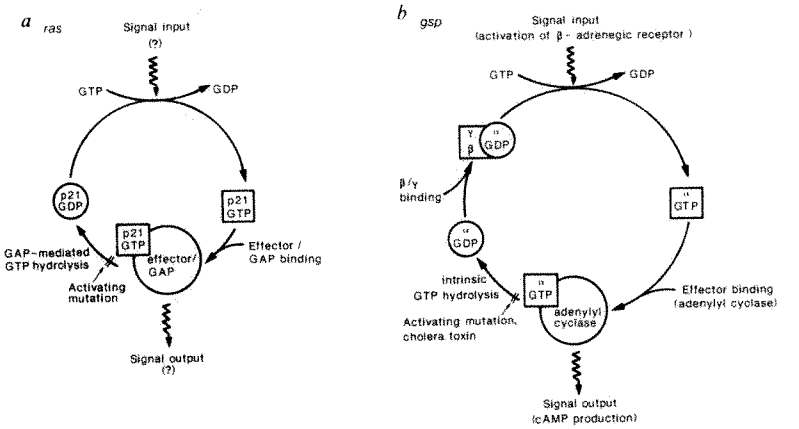

FIG. 2 Roles played by the p21(*a*) and $G_{\alpha s}$ (*b*) GTP-binding proteins in signal transduction.

G_{as} protein that would affect GTPase indirectly.

An alternative possibility is that Arg 201 is actually part of the GTPase active site of G_{as}. This would be consistent with the effects of mutagenesis or ADP-ribosylation at this position, if not with structural predictions based on p21. Pursuing this possibility, we reasoned that GAP might directly contribute to the GTPase active site of p21. We therefore searched GAP sequences for similarities with the G_{as} 201 region. When such a sequence was identified (G. Martin, Cetus), it was found to be in a region of GAP which is similar in sequence to the protein encoded by the yeast gene *IRA1*. This gene down-regulates the function of *RAS2*, a *ras*-like gene in yeast, possibly by stimulating GTPase activity[8]. Indeed, human GAP can replace the IRA1 protein in yeast (R. Ballester, personal communication). It is, therefore, possible that these conserved regions of GAP and IRA1 are sites of interaction with *ras* and *RAS2* proteins, and that for GAP at least, this region includes amino acids that contribute directly to the GTPase site. Clearly this possibility can be tested by mutagenesis in this region of the gene encoding GAP. For example, alteration of GAP residues Arg 894, Val 895 or Phe 901 might prevent GAP-mediated p21 GTPase activity.

Apart from raising questions concerning the relationship between Gsp, *ras* and GAP and focusing attention on the 201 region of Gsp/G_{α}, the work of Bourne and colleagues has two other important implications. First, the consequences of constitutive cAMP production can now be investigated in the context of cellular transformation. For example, transcription of many genes (including c-*fos*) is controlled by the action of cAMP-dependent kinases. Production of some of these gene products may be essential to malignant transformation. Proteins that control progression through the cell cycle are themselves controlled by phosphorylation. Cyclic AMP may have direct or indirect effects on these events. It may therefore be possible to trace pathways involved in oncogenesis beyond the second messenger in these cells.

Second, the possibility that other G proteins may be activated by point mutations must be considered. Most cell types do not proliferate in response to cAMP, so the spectrum of tissues reponsive to Gsp may be limited. However, the effects of G_i, G_o, G_z and others (reduced cAMP, increased phospholipases, altered ion flux and so on) may contribute to transformation in many other cell types. The oncogenic potential of these pathways has been revealed by reports that muscarinic receptors are mitogenic in neural cells (these receptors work through a G_i protein, see Hanley's News and Views article[9]), that ectopic expression of the serotonin-1c receptor (which is coupled to an unidentified G protein) is oncogenic[10], and the identification of the *mas* oncogene as an activated receptor[11]. In each of these cases it is possible that activation of the G protein, rather than of the receptor, could lead to malignant transformation. We may therefore expect to see a new wave of oncogenes, many with restricted cell specificity, and some with known biochemical functions. The pathways that these proteins control are certain to interact with those controlled by more familiar oncogenes, and should help the painstaking process of identifying biochemical events that are critical to oncogenesis. □

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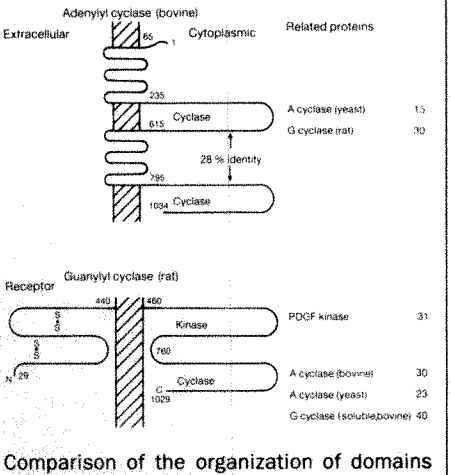
CYCLASE ENZYMES

Deciphering the mosaic

N. Michael Green

HOT on the heels of the first sequence of a guanylyl cyclase[1], has come that of adenylyl cyclase[2]. Its protein sequence, deduced from the DNA sequence, reveals an unexpectedly large proportion of hydrophobic transmembrane segments clustered in two groups of six, each alternating with 300 residue cytoplasmic domains, which show 30 per cent mutual identity, and a similar degree of resemblance to the family of guanylyl cyclases.

Such a mosaic of loose correlations is typical of many receptor proteins, but although adenylyl cyclase is membrane bound, it is not thought to be a receptor and it does not respond directly to hormonal stimuli. Elucidation of the pathway of activation of adenylyl cyclase via a separate membrane-bound receptor and a soluble cytoplasmic G-protein complex (see Fig. 2*b* of McCormick's News and Views article, opposite) has followed from the initial observations of Rodbell *et al.*[3,4]. Progress in understanding the role of G proteins was accelerated following appreciation of their involvement in the responses of rhodopsin to light. The new sequence brings with it the possibility of describing the coupling of receptors to adenylyl cyclase in similarly detailed molecular terms, especially if the cyclase can be expressed and studied by mutagenesis.



Comparison of the organization of domains in membrane-bound adenylyl and guanylyl cyclases. Percentage identities to domains of other proteins are indicated on the right. The guanylyl cyclase receptor domain has 33 per cent identity with the atrial natriuretic peptide receptor. PDGF, platelet-derived growth factor.

The history of guanylyl cyclases has followed a different but almost equally tortuous course[5,6]. Coupling of receptors to the cyclases is direct; G proteins are not involved. The recently cloned guanylyl cyclase from rat brain[1] has only a single transmembrane segment which links it to a receptor for the atrial natriuretic peptide (see figure). Between the catalytic domain of the cyclase and the membrane is a domain that resembles the oncogene-associated tyrosine kinases, though it has not yet been shown to function as such.

More detailed examination of the sequence of adenylyl cyclase shows that the pair of similar cytoplasmic domains resemble the catalytic domains of membrane-bound and soluble guanylyl cyclases more closely than they resemble the adenylyl cyclases of yeast and prokaryotes (see figure). The two clusters of transmembrane segments are similar to each other in overall content of polar residues (8–10 charges or amides) but there is no parallel between the locations of the charges nor between the lengths of loops between the segments.

The cyclase domains of all the kinases show significant similarity of sequence and a predicted secondary structure in which helices and strands tend to alternate, though not in any regular fashion. There is no indication of any sequences or secondary structure patterns characteristic of nucleotide-binding domains, such as those found in the protein-kinase domains. Krupinski *et al.*[2] draw attention to an adenylyl cyclase sequence, GAGESG, that is similar to the conserved first loop of

a nucleotide domain, but as this segment is part of the long, glycine-rich, extracellular, amino-terminus, which has no strands or helices in the neighbourhood, it cannot have anything to do with nucleotide binding. It is a common error to assume that the sequence of a short segment is sufficient to define a potential nucleotide site. Sequence and/or secondary structure should match over at least 100 residues before these assignments can be made with any confidence[7,8].

Neither of the cyclases has yet been expressed in a functional form. Nevertheless, their sequences enable one to ask much more precise questions about function and control, and to search for similar features in other proteins. A duplicated structure, similar to that of adenylyl cyclase, has been observed in the multidrug resistance P-glycoprotein[9], where the transmembrane domains may function as a transport channel. Krupinski *et al.* suggest[2] that the transmembrane domains of adenylyl cyclase might be required for export of cyclic AMP following its synthesis by the enzyme and performance as an intercellular signal, but direct evidence is lacking. Hydroxymethyl glutaryl CoA reductase is another enzyme that combines a catalytic domain with an extensive transmembrane domain, but no clear function has been found for the latter either. □

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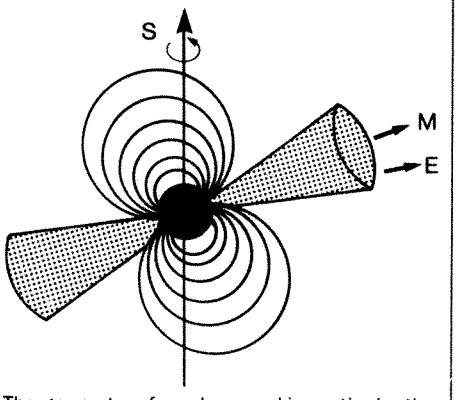
Rocket-propelled neutron stars

How can neutron stars, which we observe as pulsars, acquire velocities of 300 km s^{-1} or more? These high velocities, or rather their transverse components as seen projected on to the sky, are measured either geometrically as proper motions or indirectly by observing the rate of fluctuation of their radio signals as the pulsars move through the ionized interstellar medium. The origin of these high speeds may be either at the birth of the neutron star or later, when they are acquired through a rocket acceleration process (see figure). P. Yu. Pskovsky and



The geometry of a pulsar, and in particular the direction of the spin axis in relation to the direction of the Earth E, can be deduced from the characteristics of the radio beam, which is centred on the magnetic pole M.

O. F. Dorofeev propose elsewhere in this issue (*Nature* 340, 701–702; 1989) a test for distinguishing these two and show that the evidence favours a rocket process.

The test is based on the reasonable assumption that the direction in which a rocket acts is associated with the rotation axis of the pulsar. Fortunately we do have some good information on the direction of the rotation axis. The detailed characteristics of the radio pulses, and in particular their polarization and width, depend on the angle between the rotation axis and the line of sight which should determine the projection angle between the rocket and the plane of the sky. A reasonable body of data already exists for a correlation to be sought between the observed proper motion and the inclination of the rotation axis. If the acceleration theory is correct, the observed component of the velocity should be greater for large inclinations.

A positive result would be a surprise, as a related test involving direction of proper motion rather than amplitude has already failed to find any correlation (B. Anderson & A. G. Lyne *Nature* 303, 597–599; 1983). Pskovsky and Dorofeev do indeed surprise us by showing that there is a good correlation between velocity and the inclination of the axis. The two sets of data which are found to be correlated, although uncomfortably small, are obtained in entirely separate observations and could not be accidentally related in any indirect way. They are deductions from some difficult measurements and we must of course check the analysis on another set of pulsars.

If the correlation is proved to be good we have an answer to a question which has been with us since 1975, when E. Tademaru and E. R. Harrison (*Nature* 254, 676–677) first proposed the rocket mechanism. The remaining problem would be to explain how a single jet, or an asymmetrical pair of jets could be produced from a spinning neutron star, which we usually think of as a simple sphere with a dipolar magnetic moment. F. GRAHAM-SMITH

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DAEDALUS

Film fun

SOAP-FILMS are the most surprising and non-intuitive of liquid structures. What a pity, says Daedalus, that they are so easily burst by mechanical damage! He now proposes a way of making them really robust.

Long-chain polymers are often added to soap-film solutions to increase their viscosity and hinder gravity-thinning. And some long-chain polymers can be made as molecular closed loops. So, says Daedalus, consider a soap-film thickened with such a polymer, and punctured at a point. The momentary breach must widen catastrophically under the surface-tension of the film — unless a closed-loop molecule happens to surround the initial hole. Then the hole can expand only by breaking the loop. Daedalus calculates that just one molecular loop-molecule about a micrometre across could withstand the pull of surface-tension, and save the soap-film. It would merely have a tiny pin-hole in it.

Even better, the pin-hole would soon heal up! In its random Brownian wriggling, the loop-molecule would sooner or later close up or cross itself, spreading the film once more over its central vacancy.

So DREADCO's chemists are formulating special soap-film liquids containing molecular-loop compounds. These big rings are so hard to synthesize that the first trials are using plasmids borrowed from DREADCO's molecular biology department. Fortunately it only needs a few parts per million of loop-molecules in a film to ensure that any puncture will find itself enclosed by at least one of them. Unlike traditional frail soap-films, this new film will be totally resistant to mechanical assault.

Thus will be born DREADCO's 'hyperfoam', a resilient unbreakable air-filled cleansing foam with excellent energy-absorbing properties. Ordinary fire-fighting foam-blowers could rapidly generate it in vast amounts. People could safely leap into it from the upper stories of a burning building; aircraft landing in emergency and racing cars out of control could plough into it and be safely dragged to a stop. The military would love it as a sort of anchored smoke-screen, and the police could use it as a humane way of discouraging riots and street clashes.

Immersion in Hyperfoam could be a quite sensual experience. If made isotonic with human lung fluid, it could be breathed safely and even pleasantly; it would diffuse light, deaden sound, and retain heat. A full-submersion Hyperfoam bath, or even a whole Hyperfoam room, combining hygiene, warmth, and mild sensory deprivation, would be an appealing luxury. And Hyperfoam thinned below a light-wavelength to the invisible 'black-film' condition, would seem like a warm, sticky, viscous, caressing air. David Jones

Dissipation in computation

SIR—All proposals for 'reversible' or dissipation-free computation[1] seem to me to have a common flaw: the discussion concentrates on what happens between the input and output of a gate (or a computer), but neglects what occurs at input and output.

Taking the billiard-ball model as an example, to initiate its operation requires the simultaneous start at t_0 of several balls from their respective input channels (which determine the shooting direction). To this end the balls have to be inserted some time before t_0 and held in place against thermal motion. This can be achieved by static friction between balls and tubes or by some kind of bolts placed in front of the balls and held in place by static friction so that they cannot give way prematurely by their own thermal motion. Equally, the driving springs have to be loaded before t_0 and secured behind the balls by appropriate bolts. Even neglecting all the energy dissipated during these preparations, to initiate our computation at t_0, we have to withdraw all the bolts against static friction. This has to be done rapidly, or else t_0 would be indeterminate, and so unavoidably we have to dissipate at least several kT of energy per input ball.

Equal amounts of energy have of necessity to be dissipated at every output. Any gadget that does not yield a recognizable output signal cannot be considered to be a valid computer model; a billiard ball that is elastically reflected at the 'output' without any loss of energy cannot yield any kind of output. Whether the balls, running against reflecting springs, are trapped at the reversal point by appropriate bolts — so that they can serve for readout before they are let loose on their return trip — or whether they shift small signal-bolts at the output (which can be identical to the input-bolts of a subsequent gate), one cannot avoid the dissipation of several kT of energy per bit.

As for those models in which it is claimed that frictional losses could be reduced at will by simply reducing the speed of the balls correspondingly, when the forward-directed motion becomes slow compared with the average thermal motion, the passage of the balls from input to output becomes completely unpredictable; such a device is surely not a valid model for a computer.

Landauer[1] describes a Fredkin gate in which a ball pushes the two halves of a split control pipe apart. This ball must exert pressure on these control plates, and so will unavoidably experience static friction proportional to this pressure and independent of its velocity during its passage through the control pipe. The 'ideal viscous fluid', in which the whole affair is assumed to be immersed, will have no effect; it will simply be driven away from between ball and plates. How can such a device be claimed to function without minimum energy dissipation?

Finally, I do not believe that 'unwinding the program' can reduce the energy dissipation that occurs when resetting the output register. No ball can arrive at the output that was not started somewhere as an input ball; therefore, the numbers of input and output balls being equal, it can make no difference whether we discard these balls from the output register or, after unwinding the program, from the input register. Landauer's proposal to avoid resetting losses altogether by (hopefully) dissipation-free copying and uncopying of the input is nothing but the simple transmission from the register into a store of (necessarily) infinite capacity. Now consider the energy required to produce all this hardware.

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LANDAUER REPLIES—Reversible computation tends to be counterintuitive at first exposure. Since its original description[2], the concept has been elaborated by many with differing viewpoints, including R. P. Feynman[3]. Despite this widespread acceptance, criticism still appears in print, typically without citation of earlier similar debates. My rebuttal[4] of such a critique cites some of these previous debates. There are various possible realizations of reversible computations described in the literature: Biedermann focuses exclusively on the billiard-ball and Fredkin-gate versions. It is conceivable that one or other of the existing embodiments is flawed: demolishing one version is not an adequate rebuttal to reversible computation itself.

Biedermann focuses on the input and output operations in the billiard-ball model. As I have stressed in ref. 4, information transfer at input and output need not differ from that occurring within the computer. On the other hand, bit transfer at either end can easily be made more dissipative than that within the computer. Biedermann discusses such alternatives but does not tell us why he considers them to be optimal processes.

Most, but not all, reversible computers move back and forth in a diffusive fashion if watched over a short period, but with a predictable velocity over a long period, analogous to that of a brownian particle moving in a force field. Biedermann claims that "such a device is surely not a valid model for a computer". In my opinion, a system that carries out a computation is a computer. If the computer is going to dissipate much less energy than in current technology, it is likely to differ substantially from conventional systems.

Biedermann's charges that I ignore static friction can be answered on four levels. First, we are following the time-honoured example in the discussion of thermodynamic cycles. Second, not all reversible computers depend on mechanical devices; Bennett[5] has proposed one based on genetic-code machinery, and Likharev's[6,7] version uses Josephson junctions. Third, in these discussions, we inquire about the limits imposed by physics, and not about what can be practically realized. We are not far from the capability to build devices with atomic precision and with perfectly periodic surfaces, preserved by operation at low temperatures. There will then be no static friction. Last, even in the absence of a lubricating film, and with irregular surfaces, I believe that there is a wide range of conditions that eliminate static friction, as long as the velocity is controlled (rather than the force) and is kept small relative to thermal velocities. For ordinary macroscopic devices such a velocity is uselessly small.

Biedermann points out that the number of information-bearing degrees of freedom is conserved during reversible computations. Both the number of 'balls' and the information in the output reflects that in the input. But in the input most of the information is in the form of a supply of balls in standardized states; the variable information is confined to the program. After a long computation the final state of a reversible computer has many more balls whose state depends on the computation that has occurred.

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Space sickness on Earth

SIR—We report here the surprising after-effects of prolonged centrifuge runs in which we, the three scientist-astronauts on board the D-1 Spacelab mission (US Space Shuttle Challenger flight, 30 October–6 November 1985), have participated. We think we can simulate the space adaptation syndrome, better known as space sickness, on Earth. Such a method has, to our knowledge, not previously been reported, nor has a correlation been found between individual susceptibility to space sickness and Earth-bound motion sickness. For these reasons, it has been difficult to study the cause of space sickness or to develop preventive measures

involving selection, training and/or medication.

To be sick during the first days in space is quite normal. More than half of the astronauts are sick initially, but after two days almost all of them feel better[1]. Space sickness and adaptation are of both operational and general scientific interest. Space sickness can play a particularly negative role during short missions of a few days, and can also be hazardous during early extra-vehicular activities (in space suits).

Generally speaking, its cause is related to the absence of gravity. The study of space adaptation may therefore provide greater insight into our vestibular system, our posture, our visual reflexes and the interactions between them. Quite extensive research into these areas has already been conducted on the ground as well as in space[2,3].

The method we report here was arrived at by chance. We were investigating the effects of prolonged acceleration on humans to 2 or 3 *g*, based on an experimental finding that the immune system is more active at above normal *g* and almost inactive at 0 *g* (ref. 4). As prolonged centrifuge exposures are not reported in the literature, we started carefully with extensive medical monitoring. To limit the stresses on the body, the supine position was chosen. It was this orientation relative to the acceleration vector that led to the surprising after-effects and the similarity to space sickness.

We all then participated in more systematic tests[5] in which each of us was exposed to 3-*g* acceleration for 1½ hours while lying on our backs. The very specific symptoms induced persisted for 5–6 hours. Even very slight head movements in pitch led to a strong sensation of movement, which was provocative. All three of us perceived the readaptation to the normal 1-*g* environment when coming from the 3-*g* environment in the centrifuge to be very similar to the adaptation to 0 *g* during the first hours of our Spacelab mission, although in space both pitch and yaw head motions are provocative. Our individual susceptibilities to the space-adaptation syndrome were also reproduced. It is important to note that the full range of symptoms, from 'not being sick' to 'acute sickness', was experienced.

We conclude that this method of prolonged 'loading' of a subject with an acceleration of 3 *g* opens up a new field of vestibular research, because of the specific nature and the reproducibility of its effects. It is the first test in which the participants have observed strong similarities to their experiences during their initial hours in space. We found a strong sensitivity to prolonged acceleration in the fore-and-aft direction. It could therefore be relevant to note that all astronauts and cosmonauts have been launched in the supine position, which could have enhanced the space-adaptation problems very early in the mission.

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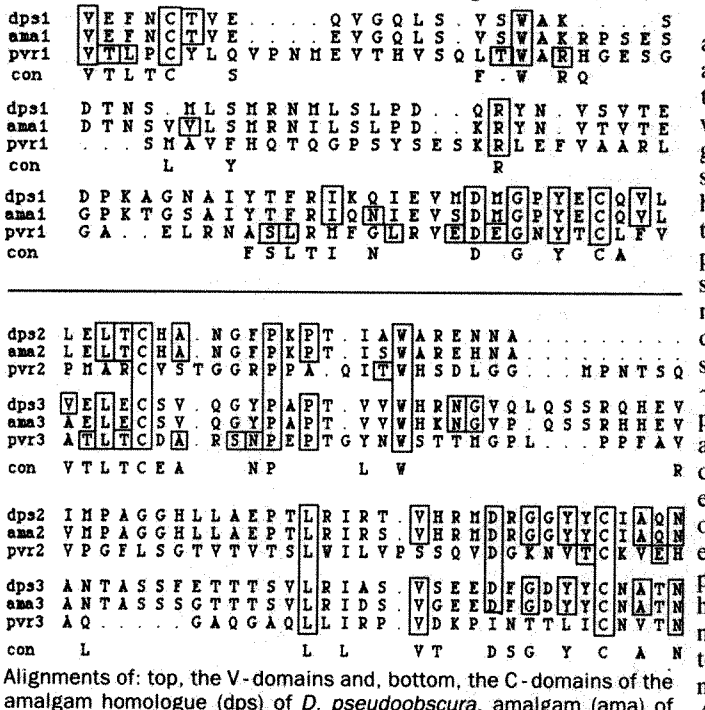
Amalgamated sequences

SIR—We have noticed an interesting similarity between the recently determined sequence of the poliovirus receptor[1] and the sequences of two other cell-surface proteins, amalgam from *Drosophila melanogaster*[2] and its homologue from *D. pseudoobscura* (T.C.K., unpublished sequence).



Alignments of: top, the V-domains and, bottom, the C-domains of the amalgam homologue (dps) of *D. pseudoobscura*, amalgam (ama) of *D. melanogaster*, and the poliovirus receptor (pvr). Residue numbers for V-domains: dps1, 45–127; ama1, 42–121; pvr1, 43–117. Residue numbers for C-domains: dps2, 153–208; dps3, 243–307; ama2, 157–212; ama3, 247–311; pvr2, 162–225; pvr3, 262–316. Boxed residues are those which are present at least three times in the immunoglobulin superfamily alignment of ref. 3; con is a consensus sequence corresponding to residues present at least four times in the immunoglobulin superfamily alignment.

Both *Drosophila* proteins have the characteristic fingerprints of the immunoglobulin fold[3], containing a variable (V)-domain followed by two constant (C)-domains. The Align program[4] gave a similarity score of close to 9 s.d. (and amino-acid identities of 24 % and 22 %) for alignment of the amino-acid sequence of the receptor with the two *Drosophila* sequences. We find strong reason to argue, therefore, that the poliovirus receptor is part of the same new class of immunoglobulin superfamily proteins that contain this arrangement of immunoglobulin-like domains.

The figure shows an alignment of V- and C-domains of the three proteins, with the immunoglobulin-fold consensus residues highlighted, obtained by the Align program and inspection. Alignment of the V-domains gave a similarity score of ~7 s.d. All three proteins contain an amino-terminal domain with properties characteristic of signal sequences[5], but only the poliovirus receptor has putative transmembrane and C-terminal cytoplasmic domains. Amalgam is expressed on *Drosophila* neuronal cells, and the poliovirus receptor is probably expressed on human motor neurons, as these are destroyed on invasion of the central nervous system by poliovirus, resulting in paralysis. These observations suggest that the two proteins have related functions, probably in cell-surface recognition events.

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Ladies of inequality

Janet Browne

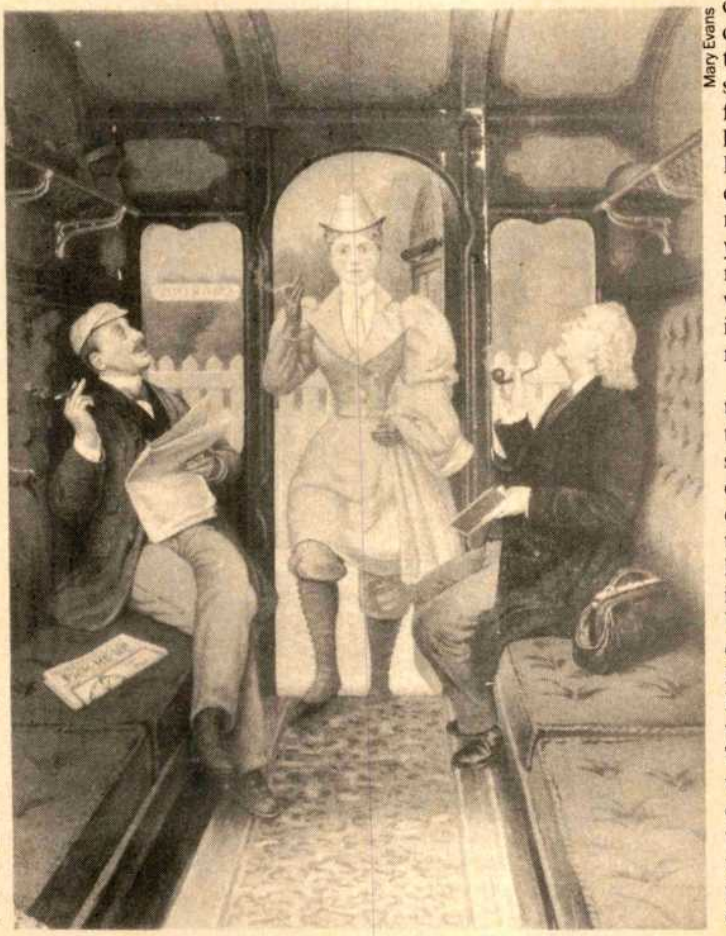
Sexual Science: The Victorian Construction of Womanhood. By Cynthia Eagle Russett. *Harvard University Press: 1989. Pp. 245. $25, £15.95.*

DESPITE the racy title, *Sexual Science* is a serious book about a serious subject. Cynthia Eagle Russett is professor of history at Yale University, and author of a number of studies on the relations between science and society in nineteenth-century America. Previously, she has focused on the way that non-scientists absorbed the implications of darwinism and the idea of thermodynamic equilibrium.

In this new book, she turns to a more complicated intermingling of worlds and takes a long, hard look at nineteenth-century science itself in order to chart the diverse and often inconsistent views that were expressed in both Britain and North America about the physical and social state of women. Many readers will perhaps be grateful that no modern axes are publicly ground, although the author is fully conversant with the latest work in womens' studies. But this does not mean that her story is a happy one: on the contrary, it fully justifies her claim that scientific work on sex differences during the Victorian period was neither unbiased nor value-free.

Philosophers, scientists, social theorists and the ordinary people in the nineteenth-century streets were all united in their belief that women were significantly different from men. Eagle Russett describes how these simple beliefs about basic biology were turned into major scientific concepts. The first section of the book, primarily following the work of Stephen J. Gould on the mismeasurement of skulls, is concerned with the way obvious anatomical differences were interpreted. The anthropological system of measuring skulls was used by biologists to show that the size of the female brain case was smaller than that of males (despite considerable evidence to the contrary), a conclusion that was thought to confirm the mental inferiority of women. Similarly, the popular science of phrenology codified all sorts of differences in the mental faculties of men and women.

These structural observations were supported by a wide range of physiological findings. Taking up one of the great nineteenth-century achievements in the study of physiology, biologists such as Patrick Geddes and J. Arthur Thomson defined men as 'katabolic' entities, metabolically active, and women as 'anabolic', passive and constructive. On another intellectual plane, Darwin worked strenuously to answer the question of why women were not as hairy as men — presumably fingering his own patriarchal beard as he did so.



Mary Evans

The 'New Woman' of the 1880s and 1890s — "upbraided for over-developing her mind to the detriment of her role as a mother . . .".

Evolutionists began to think that women were 'undeveloped' men, arrested on the way to some supposed better state. Doctors found that women were more 'nervous' than the opposite sex, prone to a whole range of mental diseases that did not afflict men (Eagle Russett misses the chance here to talk about hysteria — literally, a mental state caused by disorders of the womb and by definition found only in women).

Sexual Science is particularly good in explaining the idea of a division of labour that so clearly lies behind all these statements of difference. Human bodies were thought to mirror the body politic. Henry Maudsley and others in Britain and the United States insisted that the natural economy of the female body could not accommodate both brains and babies: the 'New Woman' of the 1880s and 1890s was upbraided for over-developing her mind to the detriment of her role as a mother (probably, as Edward Clark darkly intimated, women were graduating from college with undeveloped ovaries). Directives from the professional scientific community bombarded educationalists, demanding that they should avoid over-specialization in girls' tuition for the sake of the future of the human race. All the fears that Eagle Russett rather grandiloquently calls the "cosmic nightmare" of the closing years of the century came to rest on the idea that women without children were betraying their natural biological role.

Yet Eagle Russett leaves the pursuit of interlocking turn-of-the-century themes such as degeneration and eugenics to others: her interests expressly lie in mapping the construction of a particular view of women rather than in its possible consequences. Within this particular view, it ought also to be said that Eagle Russett is class-specific. Her book is concerned only with middle-class women. There is no indication of whether scientists had any comparable theories about working-class women, or whether the mass of working people was beyond scientific concern. Equally, one wonders what effect Queen Victoria had on the theories of men who thought women were not made to rule. Moreover, apart from a passing reference to the 'Hottentot Venus', a famous fairground exhibit in Napoleonic times, women of diverse racial origins are not discussed. It would have been illuminating to have some parallels, if any, drawn with the state of science and society in France, for example.

But this is a useful text, full of interesting ideas and pertinent examples. It will add considerably to our understanding of worlds that have gone before. □

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Diversity and Division

Roy G. Burns

Mitosis: Molecules and Mechanisms. Edited by J.S. Hyams and B.R. Brinkley. *Academic: 1989. Pp. 350. £45, $89.50.*

In the preface to this timely book, the editors comment that they have been told "that this ... will be the last book on mitosis ... before everything is understood". By contrast, Dick McIntosh notes that "Mitosis is a tough nut to crack. The people lucky enough to have found an interest in this subject will not soon be out of work." Ten chapters separate these comments and amply illustrate why they are not mutually exclusive.

The striking feature of this collection of reviews is the stress on summarizing the available evidence, rather than the work of the individual authors. For example, Kent McDonald draws attention to the diversity in structural organization, noting that the spindles of some 280 organisms have been examined. This message is reinforced in separate reviews of the centrosomes and the kinetochores, and is a reminder that any successful model must be compatible with the observed structural diversity. The concept of a single underlying mechanism, with specific variations in different organisms, is surely valid, a view which Fernando Cabral notes is supported by the genetic evidence.

Understandably, most of the discussion of this mechanism relates to anaphase, during which the chromosomes move towards the separating poles, with emphasis on the behaviour of specific classes of spindle microtubules. It is unfortunate, despite the clear efforts of the editors to cross-reference the separate contributions, that some topics, such as the polarity of the microtubules and microtubule capture by kinetochores, are duplicated in successive chapters.

The concentration on anaphase movement, both *in vivo* and using *in vitro* models, paradoxically shows how little is understood about the other stages of mitosis, such as the highly dynamic and erratic 'jiggling' of the chromosomes during pro-metaphase, or the mechanism for ensuring that the duplicated chromosomes segregate to opposite poles. The inclusion of Peter Hepler's contribution on the membranes present in spindles is a refreshing acknowledgement that microtubules may not be the only important structural component.

The book's subtitle, *Molecules and Mechanisms*, highlights how few spindle proteins have yet been characterized. For example, the centrosome of animal cells and its associated centrioles remain an osmiophilic cloud, illuminated by immunofluorescence with a variety of adventitious antibodies, yet it regulates microtubule nucleation and possibly also modulates microtubule disassembly. Similarly, the co-localization of calmodulin with some spindle microtubules, together with the evidence for local and temporal fluctuations in the free-calcium concentration, point to a fascinating yet still elusive story, while the search for molecular motors and other microtubule-associated proteins has only just begun.

Mitosis will certainly by essential reading for Dick McIntosh's "lucky people", who may not want to be reminded of those half-forgotten observations which challenge a favoured model. It should also be read by people looking for challenging problems, or who wish to be impressed by the sheer experimental inventiveness of successive generations of cell biologists.

The problem with studying mitosis is that it is a dynamic process, involving mechanisms which cannot be resolved by light microscopy. Yet electron microscopy requires the specimen to be fixed. This book is like a high-resolution electron micrograph of a mitotic spindle: although a lot of detail can be found in it, it is just a snapshot, *circa* 1989, of a dynamic process. □

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In the beginning

Beverly Halstead

Evolution and the Fossil Record. Edited by Keith Allen and Derek Briggs. *Belhaven, London: 1989. Pp.265. £25.*

On the door of my study there is a quotation from Heather Couper, borrowed in turn from *Woodstock*: "We are stardust". The magic of that phrase illuminates the consequences of the elements' evolution in the nuclear furnaces of the stars. In like manner David Attenborough conveyed the excitement of evolution and the fossil record in his television programmes *Life on Earth* and *Vanished Worlds*. John Fowles encapsulated it all: "fossils are the poetry of evolution".

With such an inherently fascinating subject how can anyone go wrong, even in a textbook? With *Evolution and the Fossil Record*, Allen and Briggs show how. Here is a book aimed primarily at advanced students of the earth and life sciences, but also at scientists in other disciplines. It is not intended to convert anyone, although there are a few snippets to treasure — Devonobiomorpha, it seems, are a new group of centipedes.

The scene is set with "Evolution of the Universe, Stars and Planets", a remarkably dense chapter which to me was incomprehensible: the diagrams, the abbreviations, the equations were quite meaningless. The table of the cosmic abundance of the elements, in which the elements are listed only by their abbreviations, is plain irritating — we should not have to remember what they all are.

"Pre-metazoan Life" is more digestible, although again densely packed (and with no discussion of the origin of eukaryotes and the pioneering contributions of Lynn Margulis). For me the pick of the bunch is "The Origins and Radiation of the Early Metazoa", which includes a few pictures of peculiar organisms. Here too, though, there are gaps in that the account of biomineralization is disappointingly brief. The fall from the Garden of Ediacara, the Cambrian explosion, is unconvincingly described as "a geologically abrupt event of great biotic significance".

Patterns of evolution and extinction in invertebrates, vascular plants and vertebrates, and catastrophes in the history of life, are all the subjects of well-turned review articles. These would have been appropriate pieces for *Palaeontology*, the journal, but not to my mind for the book's intended audience.

Two topic chapters are included, dealing respectively with the colonization of land (plants and invertebrates) and vertebrate flight (pterosaurs, birds and bats). These are popular final-examination questions, one imagines — or at least they will be in the future. The book ends with a minor piece entitled "Evolution, Creationism and Science Education", which has the same level of incongruity as the opening chapter.

Each contribution is a summary of the state of the art aimed at experts or students already deeply embroiled in the subject. Highly technical language is used throughout. The book is very tough going, and it is in this respect that I feel that Allen and Briggs have failed. If one has the tenacity to persevere, there is a great deal of interest to be found here. But the editors have demanded an unreasonable level of commitment from their readers. □

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An older Babbage

In an editorial addition to Jack Meadows's review of *The Works of Charles Babbage* (*Nature* **340**, 517; 1989), Babbage's date of birth was given as 1792, a date that is widely quoted in secondary sources. It is in fact only two years, not three, before bicentenary celebrations can begin of Babbage's birth on 26 December 1791.

Seeing things

Stuart Sutherland

Visual Processing: Computational, Psychophysical and Cognitive Research. By Roger Watt. *Lawrence Erlbaum: 1988. Pp.152. £14.95, $26.95.*

Visual Cognition: Computational, Experimental and Neuropsychological Perspectives. By Glyn W. Humphreys and Vicki Bruce. *Lawrence Erlbaum: 1989. Pp.330. Hbk £19.95, $37.95; pbk £9.95, $16.95.*

PSYCHOLOGISTS are better at discovering problems than at solving them. No sooner is a good clean theory put forward than someone rushes to the laboratory to obtain results that not only disprove it but also suggest a host of new problems demanding a totally different theory.

The late David Marr worked on a newly discovered problem, namely, how the brain obtains from the retinal image a labelled description of its luminance edges, a description that Marr called the 'primal sketch' and that must form the basis of all further visual processing. He suggested that two operations were needed. The image must be blurred, which can be achieved by taking the weighted sum of luminance over a region around a given receptor, and the second derivative of the blurred luminance distribution must be found; this function will show two departures from zero, one positive and one negative, on either side of any luminance step in the image. These operations can be achieved by filters of different sizes that respond to luminance changes over different extents. There remains the problem of how to locate the position of an edge from the output of the filters. Marr proposed that this is achieved by locating the points at which the second derivative passes through zero: as one moves across the image towards the edge of a dark figure, there will be a decrease in the second derivative followed by a rise as one moves inside the figure. Thus, the zero crossing gives the position of the edge.

In *Visual Processing*, Roger Watt describes and defends a different and highly ingenious theory developed by himself and Professor Mike Morgan. He argues that where a luminance change extends over some distance, as for example in the penumbra of a shadow, marks on the surface or noise may produce zero crossings that will mask the zero crossing produced by the overall brightness change. His solution is to sum separately all positive outputs from the different sized filters and all negative outputs. In this way the overall change in brightness will be preserved as both for the positive and the negative outputs, the points at which the resultant curves touch zero are determined only by the output from the largest filter. He argues that to detect even finer detail the largest filters are successively switched off.

Watt produces considerable evidence in favour of his theory, mainly from work on the contrast at which gratings of different widths can be seen and from the accuracy at which edges can be located. One wonders, however, just how far he has optimized the parameters of rival theories in making these comparisons, and also how far he has searched for data that disconfirm his theory.

He develops the theory to account for visual attention and the grouping of neighbouring items in the visual image. For example, on his theory a group of a dozen or so black dots randomly spaced but close together in the visual field will be immediately detected as a group because the brightness difference between the region containing them and the background will be detected by the largest filter. The following *Gedanken* experiment suggests that at the very least this is not the whole story. Imagine a group of small black and white dots on a grey background. *Prima facie*, these will be just as readily seen to form a group as a region containing only black dots, but the broadest filter will not respond since there is now no overall brightness change. Marr's theory would account for this phenomenon, since he assembled groups from the individual members rather than proceeding in the reverse direction.

Visual Processing is imaginative and stimulating, though not easy reading, partly because of the intrinsic difficulty of the subject matter and partly because Watt often does not take sufficient pains to make himself clear. *Visual Cognition* is also difficult: it is very dense, has an uneven prose style and in places the undue number of typographical errors is distracting. In so far as the book has a theme, it would appear to be the recovery of meaning from the visual image. It does not deal in depth with many of the more traditional problems, such as colour vision, depth perception, the constancies or acuity, but concentrates on the primal sketch, object recognition, motion perception, imagery, visual attention and reading. It includes not merely the experimental evidence but findings from neurological patients, a field of study that has suddenly become fashionable. Unfortunately, such findings are even more contradictory than experimental results. The authors record, for example, that in 1983 a clear case of prosopagnosia (inability to recognize faces) was recorded in a farmer, who, nevertheless, retained his ability to identify his own cows, presumably an equally difficult task. As it happens, the following year another farmer with a brain lesion was found who could not recognize his own cows but could recognize faces (a clear case of vaccagnosia in the lingo of the trade). Despite the current pursuit of neurological cases, it seems likely that brain injuries can impair any task one can think of and, of more importance, any combination of tasks. The use of such evidence for theory construction is clearly hazardous.

The hidden man — this 'picture', which originally appeared in an article by P. B. Porter in 1954, is taken from *Theories of Visual Perception*, by Ian E. Gordon, published by Wiley.

Despite the thoroughness of the reviews of each topic in *Visual Cognition*, one is left with qualms. The authors are interested mainly in the order in which different processes take place. It may well be that the existing evidence does not allow one to go beyond this, or even in many cases to reach it, but graphs containing sequences of boxes labelled "face identity units", "person identity nodes", "name generation" and so on, make one feel uneasy. It would after all be strange if one could put a name to a face without having recognized it. What one really wants to know is what goes on in each box. How are faces recognized and how are names generated?

Despite this reservation, the authors have done an excellent job in systematizing the existing evidence. The book will undoubtedly be useful as an advanced text for some years, indeed until such time as a host of new experiments has made it necessary to complicate even further their already complex exegesis. As they rightly remark, "Unfortunately the argument cannot stand there as the empirical evidence suggests a yet more complicated picture". It will continue to do so. □

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The northern light

David W. Hughes

James Joule: A Biography. By Donald S.L. Cardwell. *Manchester University Press: 1989. Pp.333. £35, $59.95.*

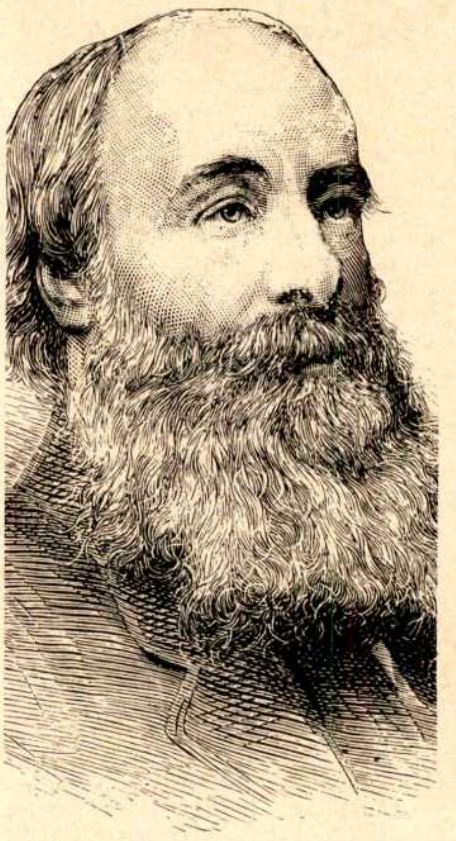
JAMES Prescott Joule, the Prescott being his mother's family name and the Joule, rhyming with cool, originating from the Derbyshire village of Youlgreave, was a Manchester/Salford man through and through. He was born on Christmas Eve 1818 into a famous brewing family and spent some of his early years working for the firm. Eventually his scientific interests predominated and it is said that the requirements of brewing technology and the accountancy needed to run a business helped to mould his scientific attitudes. A spinal weakness at birth turned him into a hunchback, and this shy and unassertive man was always sensitive about his public appearances.

Science in the time of Joule was changing from being the affair of the gentleman devotee to being the occupation of the full-time professional, ensconced in the university laboratory. Joule was in the first category. Almost all of his research was carried out in his laboratory at home and at his own expense.

According to my old science master, Joule's maxim was that "when you work you get hot". Reading Cardwell's biography one quickly realizes just why Joule is so often treated with such flippancy. His reticence often meant that his discoveries were attributed to more verbose and flamboyant researchers. Joule was a scientist's scientist. His main interest lay in exact measurement and his special genius showed itself at its best in the invention of methods for obtaining greater accuracy in quantitative experiments. He was systematic and hard-working — discreet about the origin of his ideas and cautious about his speculation.

Joule found that the heat generated by the flow of electricity was proportional to the electrical resistance multiplied by the square of the current. His experimental skills firmly established the law of conservation of energy. We take this law for granted now, but in Joule's time the complete conversion of heat into work or work into heat was no more conceivable than the conversion of gravity into hydrogen or hydrogen into gravity.

There was, too, his paddle-wheel experiment, which showed that any fluid could be heated merely by agitating it. One never forgets that because of this simple fact the water that has dropped the 49m over Niagara Falls is 0.11°C higher in temperature than at the top. More prosaically, one can state (in old units surely) that the work done in raising a weight of 1 lb through 772 ft will, if converted into heat, raise the temperature of 1 lb of water by one degree Fahrenheit. The ratio between the heat and the work was termed *J*, this symbol being introduced by Lord Kelvin (William Thomson) in honour of Joule, his friend.



James Prescott Joule — a scientist's scientist. (Mary Evans Picture Library.)

Joule also noted that the adiabatic expansion of gases leads to cooling, on account of work being done against the intermolecular forces. Experimental investigation into this process, in conjunction with Lord Kelvin, then professor of natural philosophy at the University of Glasgow, led to the gradient of an isenthalpic curve being known as the Joule–Kelvin coefficient.

Joule died 100 years ago, and in this timely biography Cardwell has done full justice to his subject. Not only has he carefully placed Joule's work in the context of the scientific aspirations of his contemporaries, he has also delved deeply into a huge collection of primary material and thus produced a definitive picture of the man and his work. The end result is a book that is not only eminently readable but is also an excellent work of reference and a fascinating introduction to James Joule and to the state of British science in the mid 1800s. □

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Tidal triggering of starbursts and nuclear activity in galaxies

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Gas distributed throughout a galaxy responds strongly to the tidal field of a companion during a merger. In some cases dynamical instability will drive a large fraction of the gas into the inner regions of the galaxy. A strong burst of star formation will follow and subsequent evolution may lead to the formation of a black hole. Continued accretion of gas by the black hole may provide sufficient power to explain quasars and nuclear activity in otherwise normal galaxies.

A FEW per cent of spiral galaxies display unusual behaviour in their nuclei, as evidenced by their intense infrared output and broad emission-line spectra. Infrared luminosities $>10^{12}\,L_{\odot}$ have been detected (by the Infrared Astronomical Satellite, in the brightest galaxies[1-3]) to originate from regions <500 parsecs in size. Similar integrated luminosities are commonly observed in the active nuclei of Seyfert galaxies, although, as suggested by time-variability arguments, the source of the radiation is even more compact—confined to $\ll 1$ pc. It is generally believed that bursts of star formation are responsible for the infrared radiation, whereas active galactic nuclei (AGN) result from accretion of matter onto central black holes[4]. The mass required to account for the observed luminosity is not trivial, even by galactic standards. Gas concentrations in the range 10^{8}–$10^{10.5}\,M_{\odot}$ have been inferred in a number of starburst galaxies[5-10]. Total fuel masses $M_{\rm fuel} \geq 10^{8}$–$10^{9}\,M_{\odot}$ are almost certainly required to power AGN over their lifetimes, given the relative inefficiency of mass conversion into energy. In extreme cases, $M_{\rm fuel} \sim 10^{10}\,M_{\odot}$, especially if the black hole forms from the accreted material. It is unlikely that the fuel could be primordial because the inferred depletion timescales are typically much less than a Hubble time[11]; hence, an external supply seems necessary. As emphasized especially by Gunn[12], the mechanism by which this material is transported into the centres of galaxies and loses its angular momentum is poorly understood.

Here I adopt the point of view that the fuel is supplied directly as gas and that its source is the disk of the host galaxy. Simple arguments indicate that viscous processes alone are not sufficient to drive large quantities of gas into the nucleus[13]. Most models have instead relied on non-axisymmetric perturbations to the potential[12,14,15], including bars and oval distortions[13,14], tidal fields[16-19] and spiral density waves[20].

Many observations suggest that star-formation rates are enhanced during galactic encounters[21-24]. The situation for AGN is unclear: recent studies[11,25,26] contradict earlier claims[27-29] that Seyfert galaxies are frequently associated with large nearby companions. Here, I report on the initial stages of an investigation of a related, but logically distinct mechanism for triggering activity in galactic nuclei: merging of galaxies. Observations indicate that up to ~20% of Seyfert galaxies have amorphous morphologies[26], perhaps indicative of recent accretion events, and seem to possess a clear excess of faint companions[25]. Motivated by these considerations I consider mergers in which the companion is much less massive than the host galaxy. My results demonstrate that mergers can lead to the accumulation of large central masses of gas in the nucleus of the primary galaxy, even if the companion is gas free. The final catastrophic inflow results from instability induced in the gas by the decaying satellite. In

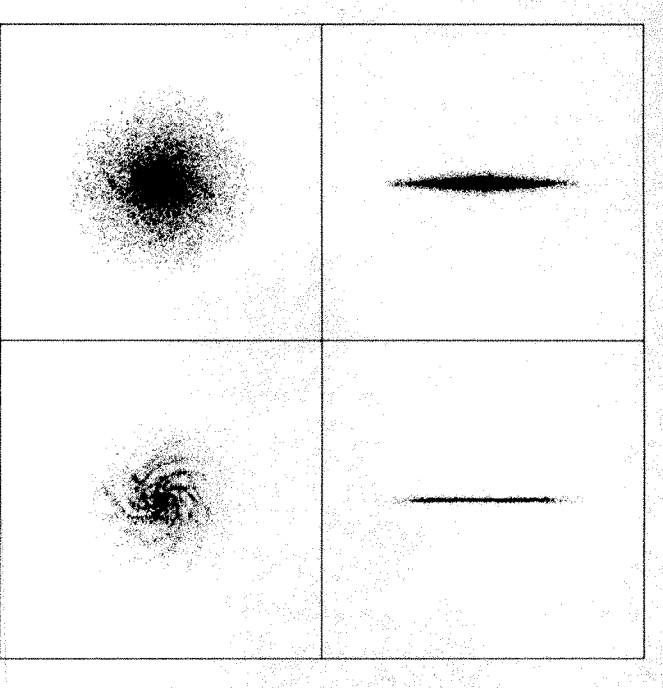

FIG. 1 Distribution of stars (top, face-on and edge-on) and gas (bottom, face-on and edge-on) in an isolated disk galaxy after five half-mass rotation periods. Here, the number of particles representing stars is $N_{\rm stars}=24{,}576$ and the number representing gas is $N_{\rm gas}=8{,}192$. The system timestep and gravitational softening parameter are $\Delta t=\tau_{1/2}/100$ and $\varepsilon=0.3$ kpc, where $\tau_{1/2}$ is the rotation period at the half-mass radius. Timesteps for individual particles can be much smaller, depending on the local properties of the system. The gravitational-force calculation is performed using the hierarchical tree method with critical opening angle $\theta=0.8$ and including terms up to quadrupole order. Each panel measures 20 radial scale lengths per edge (70 kpc).

this phase, masses $>10^9\,M_\odot$ can be deposited into the inner few-hundred parsecs on timescales $\leqslant 10^8$ yr, naturally explaining the apparent discrepancy between burst times $\tau_{burst} \leqslant 10^8$ yr and galactic dynamical times $\tau_{dyn} \sim 10^9$ yr (refs 30–32).

Method

My simulations were performed using a hybrid N-body/hydrodynamics code[33]. The self-gravity of the system is calculated using a hierarchical tree algorithm[34] that was developed and optimized for vectorizing supercomputers[35–37]. Because the tree method is gridless and has a cost that scales with the particle number as $N \log N$, a relatively large N can be used without grid-based limitations on the spatial resolution or global geometry. Hydrodynamic properties are calculated using a variation of the Monte-Carlo technique known as smoothed-particle hydrodynamics (SPH)[38–40]. SPH is fully lagrangian and represents fluid elements computationally as particles. Each particle carries along with it local thermodynamic and hydrodynamic information, including the mass density ρ, the velocity field $\mathbf{v}$ and the thermal energy u. Interpolation using a smoothing kernel permits these quantities and their derivatives to be estimated at any point in space. In my implementation, smoothing is performed with a cubic-spline kernel and the smoothing length is spatially variable[33]. Each particle has its own timestep, chosen to satisfy the Courant condition locally, so that large density contrasts can be resolved dynamically[33]. The pressure is given by the ideal-gas law $p = \rho u(\gamma - 1)$ where γ is the ratio of specific heats. The lagrangian hydrodynamic conservation laws serve as equations of motion for the particles, and include artificial viscosity terms to capture shocks and other non-adiabatic sources and sinks of energy. For the simulations here $\gamma = 5/3$ and the mean relative molecular mass is taken to be $\mu = 0.72$, as for a singly ionized gas of solar composition. Radiative effects are included by using a standard cooling curve[41], and the source terms mimic the effect of heating by cosmic rays and the formation of molecules on grains[42]. In addition, cooling is suppressed below a temperature T_c to inhibit local Jeans instability (manuscript in preparation). Here, the cooling cut-off is always $T_c = 10^4$ K. Further calculations show that the conclusions below are not limited to this choice (manuscript in preparation). Effects associated with star formation and supernova heating are ignored.

The mass distribution for the host disk galaxies is taken from the Bahcall–Soneira model for our own galaxy[43], that is, $\rho(R, z) = \rho_0 \exp(-R/h)\,\mathrm{sech}^2(z/z_0)$. The gas and stars are initially coextensive in radius with horizontal scale length $h = 3.5$ kpc and the disks are truncated at radius $R = 21$ kpc. The vertical scale length z_0 of the stars is constant across the disks and is 700 pc. Initially, the gas is distributed with $z_0 = 100$ pc and uniform temperature $T = 10^4$ K, although it adjusts rapidly and settles into a quasi-static distribution in a few cooling times. The total mass of each host galaxy is $5.5 \times 10^{10}\,M_\odot$ and the ratio of star-to-gas mass is always 10:1. The stars are given sufficient velocity dispersion to guarantee axisymmetric stability[44] with the Toomre Q parameter = 1.3 at the solar radius $R_\odot = 8$ kpc. Q is defined[45] as $\sigma_R \kappa / 3.36 G\Sigma$, where σ_R is the radial-velocity dispersion, κ is the epicyclic frequency and Σ is the surface density. The disks are embedded in non-singular isothermal haloes of scale length $a_{halo} = 3.5$ kpc and asymptotic rotation velocity $v_\infty = 220\ \mathrm{km\ s^{-1}}$. For these parameters, the rotation curve is flat beyond $R \approx 2h$ and the halo-to-disk mass ratio for $R \leqslant 21$ kpc is 3.25. The halo is rigid, but moves in response to the satellite perturbation to conserve total momentum. The companions are modelled as spherical Jaffe galaxies[46] having $\rho(r) = M_c a / [4\pi r^2 (r + a)^2]$ with scale length $a = 1$ kpc, and the disk-to-companion mass ratio is always 10:1.

Isolated disks

An example of the distribution of stars and gas in an isolated disk, after five half-mass rotation periods, is shown in Fig. 1. A spiral structure develops in both components, but is most noticeable in the gas, owing to dissipation. Because the gas remains dynamically cold, it acts to destabilize the disk and to amplify spiral perturbations. The number of arms is consistent with the interpretation that they arise from swing-amplified noise. A simple estimate in the epicyclic approximation predicts that the fastest growing mode has an azimuthal wavenumber $m = 1/f$, where f is the fraction of mass in the disk relative to the halo[47]. For the system in Fig. 1 $m \approx 4$ and a Fourier decomposition shows that this mode indeed has the largest amplitude (manuscript in preparation). Further simulations indicate that the Fourier spectrum is somewhat sensitive to the cooling cut-off parameter T_c and is broadened if the gas consists of several phases. If $T_c = 10^4$ K, as in Fig. 1, the gas is nearly isothermal and its temperature is mostly in the range $10^4\ \mathrm{K} \leqslant T \leqslant 2 \times 10^4$ K.

Averaged over the disk, the gas radiates at a relatively steady rate and the energy loss from the system amounts to ~2% of the total gas energy per rotation period. Vertically, the gas

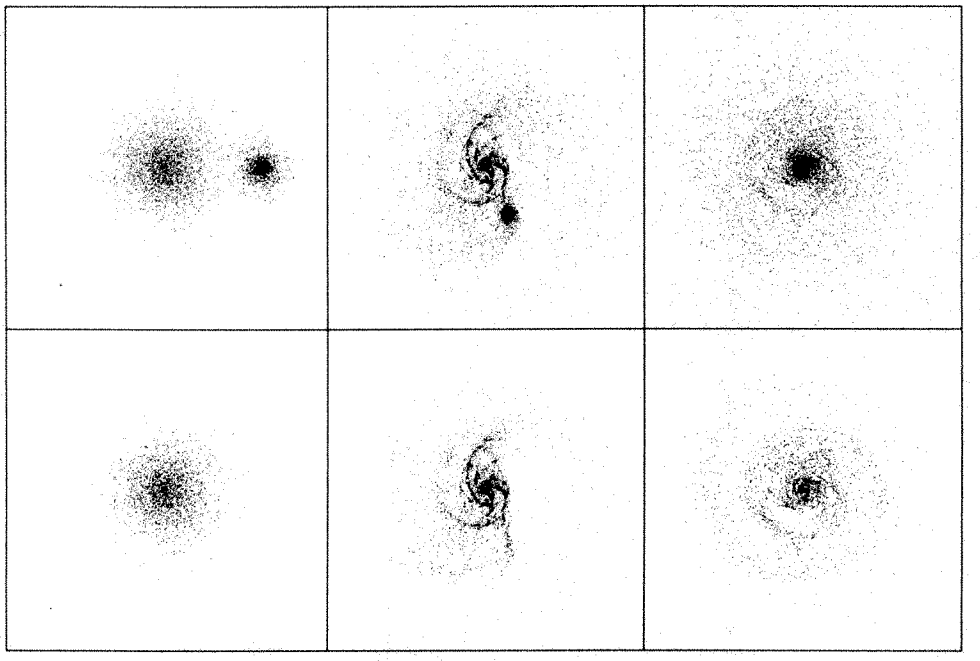

FIG. 2 Response of disk gas to decaying satellite. Top three frames show disk gas and satellite particles initially, at an intermediate time and at the final state 2×10^9 yr from the beginning of the merger. Bottom three frames show the same view of only the gas. Here, $N_{gas} = 4{,}096$ and the number of particles in the satellite is $N_{sat} = 4{,}096$. The stellar disk, not shown, consists of $N_{stars} = 12{,}288$ particles. The timestep and parameters associated with the gravitational-force calculation are as in Fig. 1 and $\varepsilon = 0.38$ kpc. Each panel measures 20 radial scale lengths per edge (70 kpc).

TABLE 1 Angular momentum of individual components

Component	$L_{1,500}$	$L_{2,000}$	ΔL
Central gas	1.6843×10^{-3}	9.6506×10^{-5}	-1.5878×10^{-3}
All gas	2.9485×10^{-2}	2.6822×10^{-2}	-2.663×10^{-3}
Disk stars	0.34500	0.35647	1.147×10^{-2}
Satellite	5.3539×10^{-2}	4.4708×10^{-2}	-8.831×10^{-3}
Sum	0.42803	0.42800	-3.0×10^{-5}

Angular momentum of individual components in merger shown in Figs 2 and 3. Central gas refers to total angular momentum of gas particles ending up in concentration at centre of disk. Remaining entries are total angular momentum of all gas, disk stars, satellite particles and the sum of these three components. Entries for each component are angular momenta at $t=1,500$ and $t=2,000$, $L_{1,500}$ and $L_{2,000}$ respectively, and the difference $L_{2,000}-L_{1,500}$. Values are given in units where the gravitational constant $G=1$, time is measured in 10^6 yr and length is measured in kpc.

remains thinner than the stars with thickness determined by the gravitational softening parameter and the temperature of the gas.

Many of these findings are in good agreement with results obtained using a 'discrete-cloud' representation of the gas[48]. This agreement suggests that the global distribution of gas in disks is only weakly sensitive to its detailed phase structure.

A merger

Here, the principal aim is to examine the response of disks, such as the one in Fig. 1, to mergers. An example is given in Fig. 2, which shows face-on views of the disk gas and a decaying satellite companion at various times during a merger. The satellite was initially on a prograde circular orbit at the edge of the disk, inclined by 30° with respect to the disk plane.

The orbit of the satellite gradually decays owing to dynamical friction. The disk responds strongly to the perturbation and shows varying structure as different internal modes become important. At intermediate times, the response is dominated by a two-armed pattern. The reaction of the disk stars to the tidal forcing is similar to that of the gas shown in Fig. 2, but somewhat less well defined (manuscript in preparation). The spiral structure in the gas is finer than in the stars because the gas can dissipate relative kinetic energy when it is compressed.

Following the merger, the disk has an overall amorphous appearance with little spiral structure evident in either the gas or stars. As first noted by Toomre[47], and later demonstrated numerically[48], this loss of spiral structure is simply due to dynamical heating of the disk stars.

Of greater interest is the accumulation of a dense central concentration of gas in the disk as a consequence of the merger, seen in the bottom panels of Fig. 2. In fact, ~35% of all the gas distributed throughout the disk initially ends up in a region with a linear extent less than 400 pc.

Radial gas inflow

The nature of the effect leading to the central accumulation of gas in Fig. 2 is most easily discerned by examining the inner regions of the disk during the final stages of the decay of the satellite, in Fig. 4. In the following discussion, time is measured in units of 10^6 yr.

As the satellite approaches the centre, its tidal field compresses both the gas and stars. The stars stream freely past each other, but the gas shocks and dissipates, forming a thin rod-like structure between $t=1,500$ and $t=1,530$. Shortly thereafter, the orbit of the satellite moves it in a direction parallel to this linear structure and the tidal field now compresses the gas along the rod, between $t=1,540$ and $t=1,550$. Eventually, the self-gravity of the gas comes into play and some of it forms a bound concentration between $t=1,550$ and $t=1,570$. The continued influence of the tidal field of the satellite and the self-gravity of the gas result in a larger build-up as the bound concentration accretes more gas. The gas blob begins to lose angular momentum to the disk stars through dynamical friction and it spirals towards the decaying satellite between $t=1,590$ and $t=1,630$. By now, the gas concentration is so massive and dense that its tidal field dominates that of the satellite. In fact, the satellite is tidally disrupted by the gas between $t=1,630$ and $t=1,670$.

The importance of the gas self-gravity is illustrated in Fig. 4, where the growth of mass in the central concentration $\mathcal{M}_g$ and the ratio W_{sg}/W_{vir} are shown. Here, W_{sg} is the gravitational potential energy of the gas blob in isolation and W_{vir} is the virial potential energy

$$W_{vir}=-2T-3(\gamma-1)U-W_{soft}, \qquad (1)$$

where T and U are the kinetic and thermal energies of this gas, respectively, and W_{soft} is a correction term that accounts for softening (manuscript in preparation). The quantities $\mathcal{M}$, W, T, and U are calculated from all the gas particles lying within 0.4 kpc of the single particle having the highest density at each time frame. Figure 4 also shows the ratio of the mass in disk stars to gas mass lying within this volume.

Nearly all the gas in the concentration accumulates between $t=1,500$ and $t=1,600$. Continued accretion thereafter increases the bound mass by roughly 10% to $\sim1.7\times10^9\,M_\odot$ at $t=2,000$. It is also clear that the self-gravity of the gas is completely negligible until $t\approx1,500$ and does not become dominant until $t\approx1,550$–$1,570$. In other words, the onset of the instability results from hydrodynamical effects and is initially driven by the dissipative nature of the gas. Note that the ratio W_{sg}/W_{vir} does

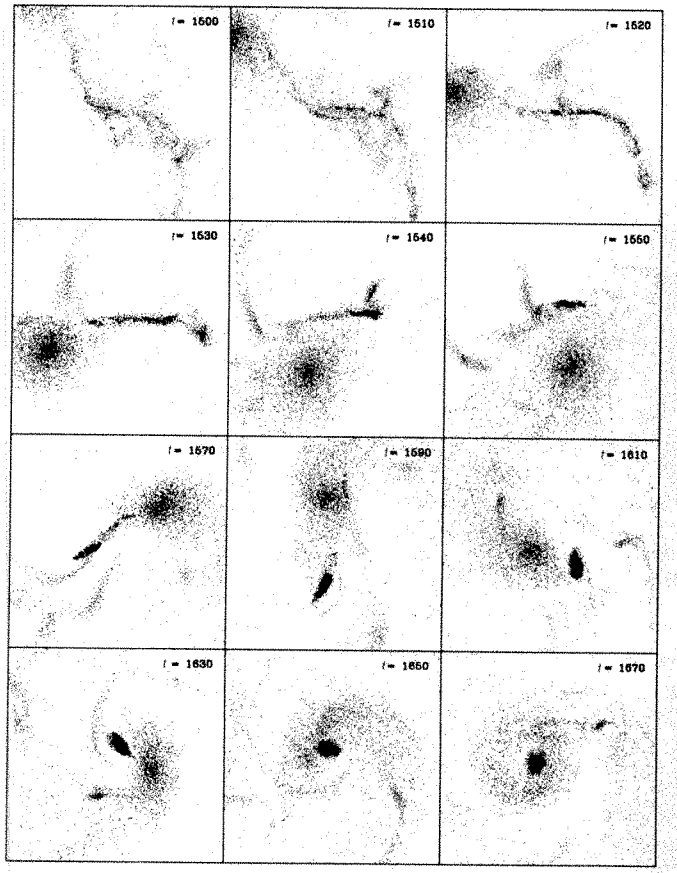

FIG. 3 Central regions of disk in Fig. 2 during final stages of merger. The disk gas and satellite particles are shown. Time, indicated in upper right-hand corner of each frame, is in units of 10^6 yr. First six panels are separated by 10^7 yr and subsequent ones are separated by 2×10^7 yr. Each panel measure 2 radial scale lengths per edge (7 kpc). Satellite is located just above upper left-hand corner of first frame at $t=1,500$.

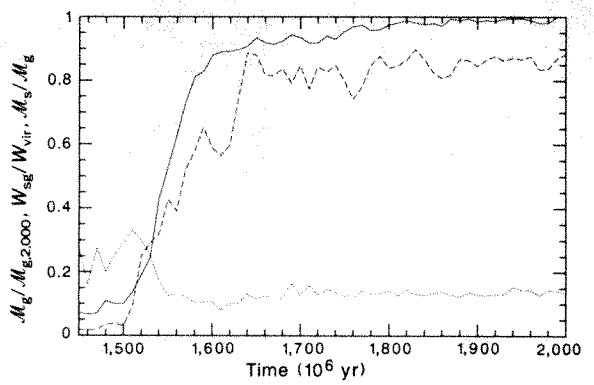

FIG. 4 Growth of mass in central gas blob $\mathcal{M}_g$ normalized to its value at $t = 2{,}000$ (solid line) as a function of time measured in units of 10^6 yr. Ratio of self-gravitating potential energy to virial potential energy for this gas W_{sg}/W_{vir} is indicated (dashed line). Importance of background potential is crudely given by ratio of mass in disk stars to the mass within central gas blob $\mathcal{M}_s$ to $\mathcal{M}_g$ (dotted line).

not approach unity exactly, as for a fully self-gravitating system, because of the background potential from the disk stars. The stars are also responsible for the rather large fluctuations in W_{sg}/W_{vir}, as shown by the ratio $\mathcal{M}_s/\mathcal{M}_g$ in Fig. 4.

Perhaps most significant is the fate of the angular momentum originally carried by the gas ending up at the centre of the disk. Figure 5 shows the time history of the total angular momentum of just the gas particles lying within 0.4 kpc of the density centre at $t = 2{,}000$. Also plotted are the total angular momentum of the gas, disk stars and satellite particles.

The net change in angular momentum from $t = 1{,}500$ to $t = 2{,}000$ for each of the components in Fig. 5 is given in Table 1. During this period, the angular momentum of the central gas decreases by a factor of roughly 17.5. The gas as a whole and the satellite particles also suffer a loss of angular momentum, with the only component gaining being the disk stars. Note that the sum of the angular momentum of the gas, disk stars and satellite is nearly constant from $t = 1{,}500$ to $t = 2{,}000$.

The central gas loses angular momentum more or less monotonically, with some irregularity only between $t = 1{,}580$ and $t = 1{,}620$. As the angular momentum of the satellite experiences a fluctuation of comparable magnitude but of opposite sign then, it seems likely that this behaviour results from tidal torques between the gas and satellite. Note from Fig. 3 that it is precisely during this period that the gas and satellite spiral towards one another.

Figure 5 strongly suggests that the primary mechanism by which the central gas sheds its angular momentum is gravitational coupling to the disk stars and satellite. Before the gas becomes self-gravitating, tidal torques are most important, but later, as the gas blob sinks to the centre, it also loses angular momentum by dynamical friction against the disk stars. To check that viscous transport is indeed much less significant, I binned the gas particles radially at $t = 2{,}000$ and calculated the time evolution of the angular momentum of the gas in each zone between $t = 1{,}500$ and $t = 2{,}000$. Only the outlying gas beyond $R > 10$ kpc acquired angular momentum—a small gain of $\Delta L = 3.3 \times 10^{-4}$, probably by gravitational coupling to the inner disk and satellite. The conclusion that angular momentum is redistributed gravitationally from the gas to the disk stars and satellite is most welcome and reassuring because the gas is only a small fraction of the total mass.

Conclusions

The results of the preceding section demonstrate one mechanism by which tidal effects can lead to the deposition of gas at the centres of disk galaxies. In the particular example presented here, a mass of gas $\approx 2 \times 10^9\ M_\odot$ is driven into a central region having an extent of roughly 400 pc on a timescale $\lesssim 10^8$ yr, which is in good agreement with observed properties of starburst galaxies. Evolution beyond that shown in Fig. 3 is uncertain because star formation and supernova heating have been ignored. Given the high densities, a strong burst of star formation is inevitable. Once the gas is self-gravitating, fragmentation and instability should lead to further radial inflow, perhaps yielding an active nucleus[4]. As the disk is distorted, but not destroyed by the merger such events may explain some Seyfert galaxies. The most promising candidates are the amorphous Seyfert galaxies[26], because of the generally featureless nature of the disk following mergers such as that in Figs 2 and 3.

The suggestion that small companions are responsible for triggering nuclear activity in some galaxies is not new and has been discussed previously[12,49,50]. The models presented here are, however, the first demonstration that mergers with satellites can deliver sufficiently large quantities of fuel to the central regions of disks to at least plausibly explain some occurrences of activity in galactic nuclei.

Further simulations demonstrate that these results are insensitive to the particle number and associated numerical diffusion, as well as to the form of the artificial viscosity. The latter conclusion is consistent with the inference that the angular momentum transport is driven mainly by gravitational rather than viscous processes. The results are clearly sensitive to the initial density profile of the gas in the disk and the structure of the companion. Because most of the gas ending up in the central concentration is initially within an inner region having a diameter of roughly two radial scale lengths, little activity would be triggered if the disk were gas-poor there. The satellite must be sufficiently dense to reach the centre of the disk, at least partially intact, before it is tidally destroyed. The calculation in Figs 2 and 3 used a satellite with a mean half-mass density twice that of the disk.

These constraints imply that merging alone probably cannot account for all occurrences of nuclear activity in galaxies. For example, the particular mechanism discussed here could not initiate activity in those early-type disks lacking gas in their central regions[51,52]. Also, it has not been demonstrated that the rate of merging with sufficiently dense companions is large

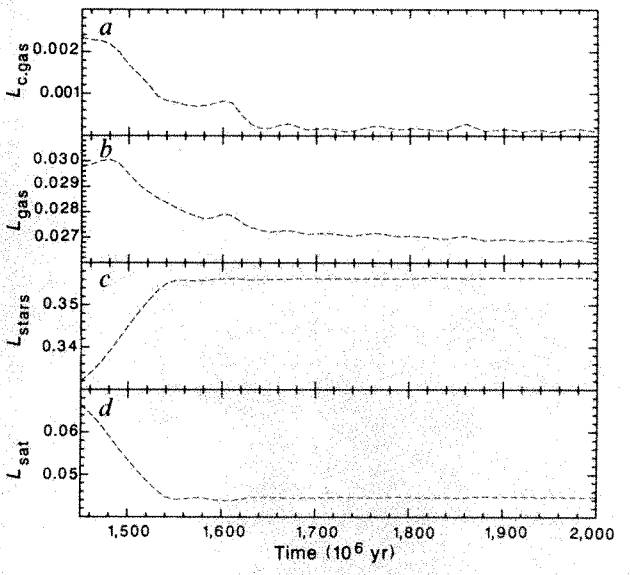

FIG. 5 Evolution of angular momenta of *a*, central gas *b*, all gas *c*, disk stars and *d*, satellite particles. Time is measured in units of 10^6 yr and angular momenta are expressed in units where $G = 1$, length is in kpc and time is in 10^6 yr.

enough to explain the observed frequency of activity. Simple calculations indicate that typical disk galaxies have accreted up to ~10% of their mass in the form of less massive satellites[53]. However, a reliable estimate of the distribution of satellite masses and densities will be possible only in the context of a complete theory for the formation of large-scale structure and galaxies.

The remaining uncertainties include the influences of the phase structure of the gas and star formation. The former is probably not critical provided that the gas can dissipate as it is tidally compressed. The typical separation between fluid elements in the gas rod that develops between $t = 1{,}500$ and $t = 1{,}530$ in Fig. 3 is ~100 pc. The gas would still be expected to dissipate strongly at this point, even if it consisted entirely of discrete clouds, provided that the cloud radii are not much less than about 50 pc. Given that giant molecular clouds have sizes of this order, it appears that the detailed small-scale structure of the gas is unimportant. Star formation will undoubtedly modify the evolution and its neglect is probably the most serious weakness of the models presented here. Nevertheless, once the gas is drawn out into a thin rod-like structure it seems likely that a large fraction of it will become self-gravitating and fuel central activity. This could be prevented only if the gas were converted to stars at an exceptionally high efficiency before this stage, leading to a more extended starburst than suggested by Fig. 3.

The relative importance of mergers, as opposed to nearby transient encounters, for nuclear activity remains unanswered. As noted previously, current observations indicate that Seyfert activity is not correlated with galactic interactions[11,25,26]. Theoretical evidence on this question is ambiguous. Noguchi has studied encounters of disks consisting of both stars and gas with other equal-mass galaxies[19]. He finds that pure parabolic collisions can induce bar instability in the stars, removing angular momentum from the gas and driving it into the inner regions of the disks. As noted by Barnes[54], however, fully self-consistent encounters of equal-mass galaxies on initially parabolic orbits typically lead to merging within a few dynamical times unless the pericentre distance r_p is much greater than the extent of the haloes r_h. This condition is probably satisfied in Noguchi's calculations because he abruptly truncated his haloes at $r_h = 20$ kpc and chose $r_p = 40$ kpc. For more extended haloes, encounters with $r_p = 40$ kpc will be highly inelastic and should lead to rapid merging. Noguchi was unable to examine such effects with his numerical approach. It appears that it will not be possible to establish a firm link between transient encounters and Seyfert activity without appealing to self-consistent models.

The scenario presented here predicts that the origin and evolution of activity in at least some galaxies is controlled by events on galactic scales. Such an interpretation is in accord with the suggestion that starbursting and nuclear activity represent different phases of the same process[30–32]. Furthermore, the frequency and intensity of activity should increase with the rate of merging and the fraction of luminous mass in the form of gas. This indeed appears to be true of observed active galaxies, which are more prevalent and luminous at high redshifts. Indeed, activity may be triggered during galaxy formation, if at least some of the mass accumulates in the form of discrete subunits. Ultimately, mechanisms like that described here may explain a much broader class of phenomena than aberrant behaviour in galaxies observed today, including quasars and extragalactic radio sources. □

Received 27 April; accepted 12 July 1989.

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ACKNOWLEDGEMENTS. I thank J. Barnes, J. Binney, J. Gunn, C. Norman, M. Rees, A. Toomre, S. Tremaine and M. Weinberg for discussion about active galactic nuclei and dynamics of gas in galaxies. This work was supported in part by a generous allocation of Cray Y-MP time from the Pittsburgh Supercomputing Center and by a New Jersey High Technology Grant.

GTPase inhibiting mutations activate the α chain of G_s and stimulate adenylyl cyclase in human pituitary tumours

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A subset of growth hormone-secreting human pituitary tumours carries somatic mutations that inhibit GTPase activity of a G protein α chain, α_s. The resulting activation of adenylyl cyclase bypasses the cells' normal requirement for trophic hormone. Amino acids substituted in the putative *gsp* oncogene identify a domain of G protein α-chains required for intrinsic ability to hydrolyse GTP. This domain may serve as a built-in counterpart of the separate GTPase-activating proteins required for GTP hydrolysis by small GTP-binding proteins such as $p21^{ras}$.

MANY oncogenic mutations promote tumour growth by inducing autonomous activity of proteins that normally transmit proliferative signals initiated by extracellular factors. The role of cyclic AMP as an intracellular second messenger for several trophic hormones and its ability to stimulate growth of certain cultured cells[1,2] predict that oncogenic mutations should be found in genes for proteins that control its synthesis. Here we report identification of mutations that cause autonomous cAMP synthesis in four growth hormone (GH)-secreting human pituitary tumours. The mutations cause constitutive activation of α_s, the GTP-binding subunit of the stimulatory regulator of adenylyl cyclase, G_s (refs 3–6), by inhibiting its GTPase. These mutations fulfill the specific prediction[7] that a subset of pituitary tumours with constitutively active adenylyl cyclase[8] carry oncogenic mutations in the α_s gene. Moreover, the biochemical properties and cellular function of α_s—unlike the products of most oncogenes—have been characterized in detail. For this reason, the locations and functional alterations produced by these tumour-associated α_s mutations significantly extend our understanding of the conserved mechanism of GTP hydrolysis in the signalling G proteins, $p21^{ras}$ and many other GTP-binding proteins. Specifically, they point to a region of α_s that is intimately involved in GTP hydrolysis and that probably acts in α_s as a built-in counterpart of the separate GTPase-activating proteins (GAPs)[9,11] required for GTP hydrolysis by other GTP binding proteins, such as $p21^{ras}$.

GH-releasing hormone (GHRH) uses cAMP as a second messenger to stimulate GH secretion and proliferation of normal pituitary somatotrophs[11]. Vallar *et al.*[8] reported constitutive activation of G_s in a subset of GH-secreting human pituitary tumours. Measurements of adenylyl cyclase activity clearly identified two distinct groups of tumours: group 1 tumours had low basal adenylyl cyclase activity that responded normally to stimulation by hydrolysis-resistant GTP analogues, AlF_4^- ion and GHRH, whereas group 2 tumours had marked elevation of basal adenylyl cyclase and responded poorly or not at all to the stimulatory agents. G_s activity was normal in group 1 tumours and constitutively active in group 2 tumours[8].

Prompted by the idea that α_s activated by mutation could relieve the requirement for GHRH stimulation of adenylyl cyclase and thereby contribute to growth of these tumours, we sought and found mutations in α_s genes of group 2 tumours.

Identification of α_s mutations

We tested eight pituitary tumours surgically removed from acromegalic patients suffering from excessive secretion of GH. Using basal adenylyl cyclase activity and its response to stimulation by AlF_4^- as criteria, we identified four group 1 tumours and four in group 2 (Table 1). As assessed by biochemical complementation of α_s-deficient S49 *cyc*$^-$ membranes[8], G_s activity was constitutively elevated (in the presence of GTP alone) in the four group 2 tumours and normal in the four group 1 tumours (results not shown).

To look for mutations in the group 2 tumours, we reverse-transcribed RNA, amplified the coding region of α_s complementary DNA using the polymerase chain reaction (PCR)[12], subcloned the amplified DNA into M13 vectors, and sequenced the entire coding region from multiple M13 clones derived from each tumour. The α_s cDNAs from all four group 2 tumours contained mutations (Table 1). Mutations in three tumours replaced Arg 201 (wild-type codon CGT) with either Cys (TGT in tumours 5 and 7) or His (CAT in tumour 6); using the single-letter code for amino acids, we designate these mutations R201C and R201H, respectively. The mutation in tumour 8 replaced Gln 227 (wild-type codon CAG) with Arg (CGG); this mutation is designated Q227R.

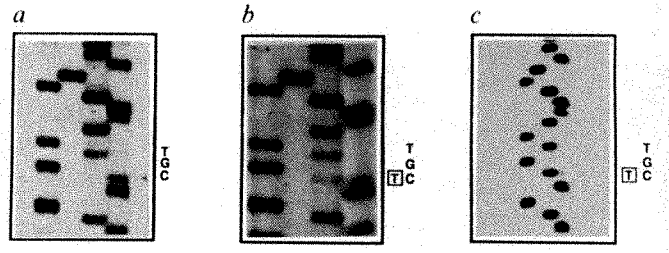

FIG. 1 Direct sequencing of PCR-amplified region surrounding codon 201. *a*, Genomic DNA from tumour 1 (group 1). *b*, Genomic DNA from tumour 5 (group 2). *c*, cDNA from tumour 5 (group 2). Lanes from left to right are G, A, T, C. The nucleotide sequence of codon 201 is listed beside each panel. The mutant nucleotide in tumour 5 is boxed. Direct sequencing was performed as described in Table 1 legend.

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TABLE 1 α_s Mutations in human pituitary tumours

Tumour		Adenylyl cyclase (pmol cAMP mg^{-1} min^{-1}) Basal	AlF_4^-	DNA	Codon 201	Codon 227
Group 1	1	13	170	Genomic	Arg	Gln
	2	6	96	Genomic	Arg	Gln
	3	16	300	Genomic	Arg	Gln
	4	43	130	Genomic	Arg	Gln
Group 2	5	170	130	cDNA	Arg (2)/Cys (3)	Gln
				Genomic	Arg/Cys	Gln
	6	480	260	cDNA	Arg (0)/His (4)	Gln
				Genomic	Arg/His	Gln
				Genomic (blood)	Arg	Gln
	7	190	130	cDNA	Arg (0)/Cys (3)	Gln
				Genomic	Arg/Cys	Gln
	8	180	120	cDNA	Arg	Gln (0)/Arg (3)
				Genomic	Arg	Gln/Arg
				Genomic (blood)	Arg	Gln

Eight pituitary tumours are divided into groups 1 and 2 by adenylyl cyclase activities. Columns on right list, for each tumour, the source of DNA (genomic DNA from tumour, cDNA from tumour, or genomic DNA from peripheral blood of the same patient) and the amino acid(s) encoded by codons 201 and 227, determined by sequencing PCR-amplified cDNA or genomic DNA. Two amino acids are listed when bases encoding both were found in a single DNA sample. Numbers in parentheses indicate the number of individual M13 cDNA clones sequenced that encoded the specified amino acid. Pituitary adenomas from patients with GH excess were surgically removed and stored at −70 °C. To measure adenylyl cyclase activity, membranes were prepared from tumour homogenates and adenylyl cyclase activities measured[8] in the absence or presence of 10 mM NaF. To perform sequence analysis of subcloned PCR amplified cDNA, total RNA was extracted from tumour homogenates as described[36]. First strand cDNA was produced in a reaction containing 7 μg total RNA, oligo(dT) primer and reverse transcriptase, according to the protocol in the AMV reverse transcriptase kit from Bethesda Research Laboratories. Four per cent of the cDNA reaction volume was amplified as described[12]. Primers were designed to match the 5′ and 3′ noncoding regions of human α_s (ref. 37), in order to amplify the complete coding region. Each primer contained an artificial restriction site at its 5′ end to facilitate subcloning. The 5′ primer (5′-GCCGGTACCCGGCCGCGCCCCGCCGCCGCCG-3′) had a *Kpn*I restriction site and the 3′ primer (5′-TTAAAGCTTTAATTAAATTTGGGGGTTCC-3′) had a *Hin*dIII restriction site. The PCR mixture contained 2.5 U DNA polymerase from *Thermus aquaticus* (Perkin-Elmer Cetus) and 25 pmol of each primer. Amplification was accomplished in 40 cycles of 1 min at 94 °C, 1 min at 58 °C, and 2 min at 72 °C, in a Perkin-Elmer Cetus Thermocycler. Amplified DNA was desalted and primers were removed by gel filtration with G-50 Sephadex spin columns (Boehringer Mannheim) and ethanol precipitation. The purified DNA was digested with *Kpn*I and *Hin*dIII and subcloned into M13mp18 or M13mp19. The Sequenase method of dideoxy sequencing was used to determine the entire α_s coding sequence from at least two M13 clones derived from two separate amplification reactions for each group 2 tumour. For direct sequence analysis of PCR amplified cDNA and genomic DNA, first strand cDNA from tumours was prepared as described above. Genomic DNA was extracted from tumours or peripheral blood by homogenizing samples in a glass-Teflon homogenizer in a lysis buffer (4 M Urea, 1% Triton X-100, 10 mM EDTA, 100 mM NaCl, 10 mM Tris, pH 8.0, 10 mM DTT, and 0.2 mg ml^{-1} Proteinase K); samples were incubated overnight, repeatedly extracted with phenol/chloroform, and DNA recovered by ethanol precipitation. PCR amplification was performed with primers flanking a region that encompassed both codons 201 and 227; the 5′ primer (5′-GTGATCAAGCAGGCTGACTATGTG-3′) was located in exon 7 and the 3′ primer (5′-GCTGCTGGCCACCACGAAGATGAT-3′) was located in exon 10 (ref. 37). Four per cent of the cDNA reaction volume or 50 ng genomic DNA was amplified as described above, except that unequal molar concentrations (12.5 pmol:1 pmol) of the primers were used to generate single-stranded DNA for direct sequence analysis, as described[38]. The reaction mixture was desalted and excess dNTPs were removed by repeated (3–4×) spin-dialysis on a Centricon 30 (Amicon). Samples were then vacuum-dried and resuspended in water. Half of the reaction product was sequenced using Sequenase. The primer used at low concentration in the amplification reaction was used in the sequencing reaction.

Both amino-acid residues replaced by these mutations were already known or suspected to be important in α_s function. Arg 201 is the residue thought to be ADP-ribosylated by the pathogenic exotoxin of *Vibrio cholerae*[13], a covalent modification that activates α_s by inhibiting its GTPase activity[14]. Gln 227 is located in the presumed guanine nucleotide binding region of α_s[15], at a position corresponding to Gln 61 of $p21^{ras}$; mutational replacement of Gln 61 in $p21^{ras}$ by almost any other amino acid produces a protein that promotes malignant transformation[16]. The functional significance of mutations at these two sites is discussed in more detail below.

To confirm and extend these findings, we determined nucleotide sequences surrounding codons 201 and 227 by direct sequencing of PCR-amplified regions of genomic α_s DNA (see Table 1 legend for details). Genomic DNA from each of the group 1 tumours showed only wild-type sequence at codons 201 and 227. Genomic DNA from each of the group 2 tumours showed the same mutation detected in the corresponding cDNA. Group 2 genomic samples also showed wild-type sequence at the same positions (example in Fig. 1; summarized in Table 1). This presumably indicates the simultaneous presence of both a mutant and a normal α_s allele and suggests that constitutive activation of α_s is a dominant phenotype (see below). By analogy with most dominant oncogenes, we expect the α_s mutations to be somatic in origin and, in keeping with this expectation, genomic DNA from peripheral blood cells of two patients with group 2 tumours contained only wide-type α_s sequences (Table 1).

Mutations constitutively activate α_s

To determine whether the mutations cause constitutive activation of α_s and how they might do so, we altered the nucleotide sequence at appropriate codons of a cDNA encoding a form of rat α_s of relative molecular mass 52,000 (ref. 17) and expressed the R201C, R201H, and Q227R mutant proteins in α_s-deficient S49 *cyc*$^-$ cells[18–21] (for details, see Fig. 2 legend). The adenylyl cyclase phenotypes of cells expressing the mutant proteins were compared with that of a clone (csw1.3) of *cyc*$^-$ cells transformed with wild-type rat α_s (refs 18, 20 and 21).

In *cyc*$^-$ cells, each of the three mutant proteins reproduced the adenylyl cyclase phenotype observed in group 2 tumours. As compared with wild-type α_s (in csw1.3 membranes), adenylyl cyclase in membranes of Q227R, R201C, and R201H cells showed elevated basal activity (measured in the presence of GTP alone) (Fig. 2). Relative to GTP alone, GTP plus isoprenaline, a β-adrenoceptor agonist, stimulated a substantially smaller

increase in adenylyl cyclase in membranes containing mutant α_s (2- to 3-fold) than in csw1.3 membranes (13-fold). AlF_4^- ion and GTP-γS, a hydrolysis-resistant GTP analogue, stimulated adenylyl cyclase in csw1.3 membranes, but not in Q227R, R201C, or R201H membranes (Fig. 2).

These results closely parallel patterns of adenylyl cyclase responsiveness observed in membranes of group 2 tumours, which converted ATP to cAMP at a markedly elevated rate in the absence of stimulatory agents. Adenylyl cyclase in these tumour membranes was stimulated only slightly by GHRH and not at all by GTP analogues or AlF_4^- (ref. 8).

In principle, the Gln 227 or Arg 201 mutations could activate α_s in two ways. Either type of mutation could mimic the normal effect of hormone-receptor complex, which activates α_s by increasing the rate at which GDP dissociates from its inactive form, thereby accelerating binding of GTP and attainment of the active state. Alternatively, mutations could inhibit the intrinsic GTPase of α_s, which serves as a timing device that turns off the protein's active GTP-bound conformation[3–6]. This second mechanism turns out to account for α_s activation by mutations at both sites, as described below.

Gln 227 mutations

Even before the Q227R mutation was identified, site-directed mutations[21,22] had shown that mutational replacement of Gln 227 by Leu (Q227L) activates α_s by inhibiting its GTPase activity. Similarities in primary structure between short stretches of conserved amino-acid sequence in G protein α-chains and parts of the guanine nucleotide-binding domain of $p21^{ras}$ had suggested[15,23] that these α-chain regions form a GTP-binding domain structurally and functionally similar to that of $p21^{ras}$. The similar effects of the α_s Q227L mutation and the cognate Q61L *ras* mutation on GTPase, and biological activity of the respective mutant proteins[16,24] confirmed that α-chains and $p21^{ras}$ share similar GTP-binding domains.

Accordingly, we examined the effect on GTPase activity of the tumour-associated α_s-Q227R mutation expressed in *cyc*$^-$ cells. The Q227R mutation inhibited GTPase activity of α_s, reducing $k_{cat\cdot GTP}$ from a wild-type value of 4.1 min^{-1} to 0.12 min^{-1} (Fig. 3). These $k_{cat\cdot GTP}$ values were estimated by an indirect method[25] based on measurements of membrane-associated adenylyl cyclase activity (Fig. 3). The genetically engineered Q227L mutation similarly reduced $k_{cat\cdot GTP}$ measured by both the indirect method[21] and by direct assay using pure recombinant protein expressed in *Escherichia coli*[22]. The very similar results obtained by the two methods[21,22] support the validity of the indirect method for estimating $k_{cat\cdot GTP}$ of α_s.

Thus phenotypes produced by the deliberately designed Q227L mutation and by the Q227R mutation found in a human pituitary tumour are consistent with the hypothesis that α_s and $p21^{ras}$ share structurally similar guanine nucleotide-binding domains and use similar or identical mechanisms to hydrolyse GTP.

Arg 201 mutations inhibit GTPase

Although they lack precedents in $p21^{ras}$ or other GTP-binding proteins, the Arg 201 mutations yield potentially important clues regarding the mechanism of GTP hydrolysis by α_s. In keeping with their effects on GTP-dependent adenylyl cyclase activity (Fig. 2), the R201C and R201H mutations inhibited GTPase activity of α_s, reducing $k_{cat\cdot GTP}$ ~30-fold to 0.12 min^{-1} and 0.15 min^{-1}, respectively (Fig. 3).

Because Arg 201 is the α_s residue whose side chain is thought to be ADP-ribosylated by the exotoxin of *V. cholerae*, we also measured the $k_{cat\cdot GTP}$ of wild-type α_s in membranes treated with cholera toxin and NAD^+ (Fig. 3). Toxin treatment decreased $k_{cat\cdot GTP}$ from 4.1 to ~0.17 min^{-1}. The effect of toxin-catalysed ADP-ribosylation of Arg 201 closely resembles the effects of replacing the same residue by Cys or His. This similarity indicates that attachment of ADP-ribose to Arg 201 inhibits GTPase more specifically than expected: perhaps the rate of GTP hydrolysis is not reduced by nonspecific steric hindrance from the bulky ADP-ribose attached to Arg 201, but rather because the specific shape and location of the Arg 201 side chain itself are necessary for normal GTP hydrolysis.

This notion predicts that replacement of Arg 201 by virtually any amino acid will inhibit GTPase. Soon after finding the R201C and R201H mutations in pituitary tumours, we learned that this prediction had already been partially fulfilled; Freissmuth and Gilman constructed recombinant mutant α_s in which Arg 201 was replaced by Ala, Glu or Lys, and in each case the recombinant mutant α_s protein, synthesized in *E. coli*, showed markedly decreased $k_{cat\cdot GTP}$ and enhanced ability to stimulate adenylyl cyclase in the presence of GTP alone (A. G. Gilman, personal communication). Duplication of the functional change by replacing Arg 201 with five different amino acids makes it quite likely that the side chain of this Arg residue interacts with or forms a part of the molecular timing device that regulates $k_{cat\cdot GTP}$. The fact that substitution of a positively charged Lys residue for Arg 201 inhibits GTPase indicates, in addition, that the postulated function of Arg 201 cannot be supplied simply by a side chain with positive electrical charge.

Activated cholera toxin is a mono-ADP-ribosyltransferase, which transfers ADP-ribose from NAD^+ to the side chain of arginine and arginine methyl ester, but not to other amino acids[26]. Consequently, we expected that the toxin would not ADP-ribosylate α_s-R201C or α_s-R201H. This prediction was not completely fulfilled: the toxin catalysed transfer of radiolabel from [^{32}P]NAD^+ to both α_s-R201C and α_s-R201H expressed in *cyc*$^-$ cells, although much less efficiently than seen with wild-type α_s (Fig. 4). Although precise estimates of the relative extent of ADP-ribosylation are difficult, owing to differing amounts of α_s protein in different membrane preparations, we estimated that

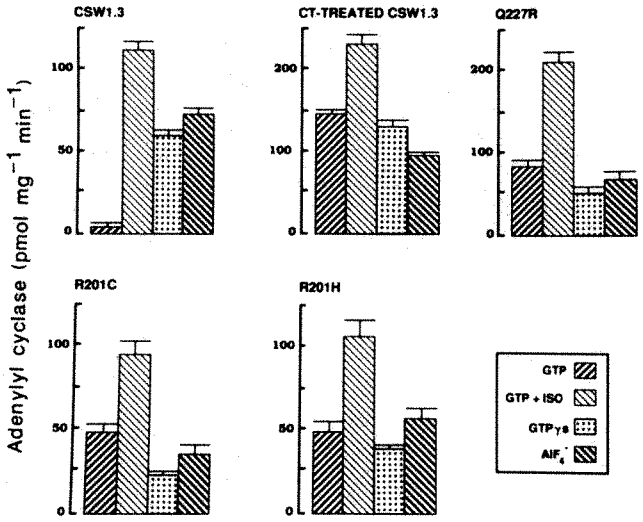

FIG. 2 Adenylyl cyclase stimulated by normal and mutant α_s. Membranes were assayed for adenylyl cyclase activity in the presence of the following concentrations of activators: 50 μM GTP; 100 μM isoprenaline (ISO); 50 μM GTP-γS; 10 μM $AlCl_3$ + 10 mM NaF (AlF_4^-). Values represent means ± s.d. for triplicate determinations.

METHOD. The Q227R, R201C and R201H mutations were introduced into the 52 K form of a normal rat α_s cDNA[17,21] by site-directed mutagenesis with the Bio-Rad Muta-Gene Phagemid *in vitro* mutagenesis kit[39]. Mutant α_s DNAs were subcloned into the retroviral expression vector MV7 and transfected into S49 *cyc*$^-$ cells as described previously[18,20,21]. Membranes were prepared from *cyc*$^-$ clones that expressed the mutant α_s proteins[21]. Cholera toxin treatment of membranes and adenylyl cyclase assays were performed as described previously[21], except that the time of incubation in the adenylyl cyclase assays was decreased from 30 to 15 min.

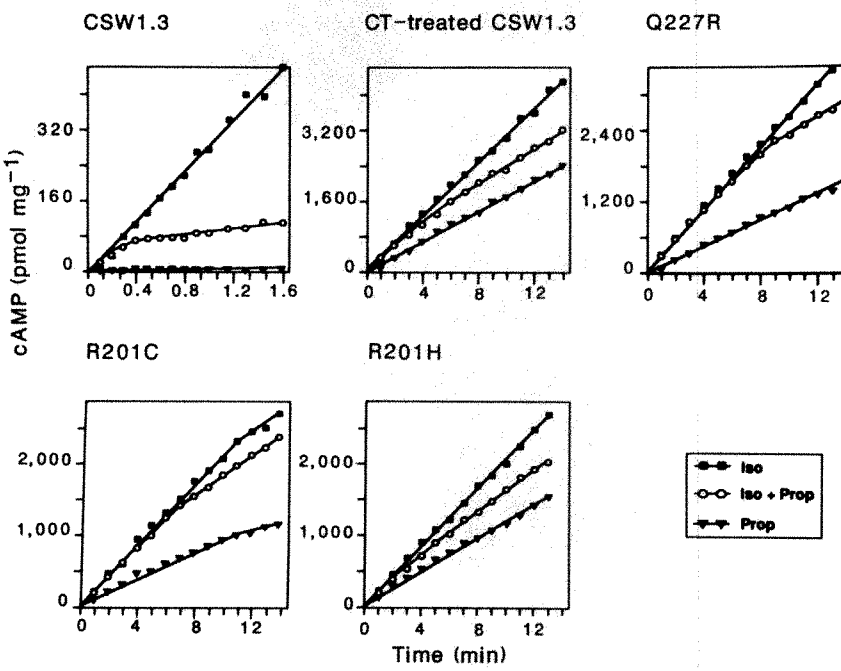

FIG. 3 Indirect measurement of GTPase. The average duration of 'active' G_s was assessed indirectly, by adaptation[21] of a method[25] using measurements of adenylyl cyclase activity. Briefly, adenylyl cyclase in membranes was first activated for 3 min by isoprenaline (20 μM) plus GTP (50 μM) in the presence of non-radioactive ATP; at time = 0, agonist-promoted introduction of GTP into the binding site of G_s was arrested by addition of a β-adrenoceptor antagonist, propranolol (50 μM). [α-^{32}P]ATP was added simultaneously with propranolol, and aliquots were removed at the indicated times for determination of [^{32}P]cAMP (see Fig. 2 legend). The rate constant for the GTPase turn-off reaction, $k_{cat\text{-}GTP}$, was calculated from the time course (Iso + Prop, ○) of the propranolol-induced reduction in the rate of agonist-dependent cAMP synthesis (down to the rate measured in the absence of agonist (Prop, ▼)). Also shown (Iso, ■) is the time course of isoprenaline-stimulated [^{32}P]cAMP synthesis in the absence of propranolol.

the toxin labelled α_s-R201C < 10% as efficiently as wild-type α_s, and α_s-R201H 10–20% as efficiently as wild type α_s (see Fig. 4 legend for details). M. Freissmuth and A. G. Gilman (personal communication) found similar results with their mutants: in each case, the toxin ADP-ribosylated R201X mutant α_s < 20% as efficiently as it modified wild type α_s.

This unexpected set of results can be explained in at least two ways. First, Arg 201 could be mistakenly identified as the amino acid modified by cholera toxin, which may normally ADP-ribosylate another Arg residue. Arg 201 of α_s has not been directly shown to be modified by the toxin. Instead, the direct experiment[13] was performed with α_t, the α-subunit of retinal transducin: α_t was modified by the toxin at Arg 174, which corresponds to Arg 201 of α_s. A second interpretation, perhaps more likely, is that the toxin's ADP-ribosyltransferase activity prefers Arg 201 but will use another residue as the acceptor when confronted with an α_s molecule lacking an Arg side chain at the correct position. In this context it is interesting that α_s (like almost all other G protein α-chains) contains another Arg residue just two positions upstream from Arg 201; perhaps this residue serves as a secondary acceptor site for ADP-ribose.

In keeping with the relatively low extent of toxin-catalysed modification of α_s-R201C and α_s-R201H, treatment with toxin and NAD^+ did not alter the ratio of adenylyl cyclase activities measured in the presence of GTP compared with GTP plus isoprenaline (result not shown).

Conditional versus intrinsic GTPases

Arg 201 is located in domain II (ref. 15) of α_s (residues 61–218). This domain is demarcated at either end by conserved signature sequences (GXXXXGK and DXXGQ) that are found in the guanine nucleotide domains of other GTP-binding proteins, such as p21^{ras} and GTPases involved in ribosomal protein synthesis, exemplified by elongation factor Tu (EF-Tu). The function of the 158 residues of domain II and of the cognate domains of similar size in other G protein α-chains, is unknown. Domain II has no obvious counterparts in p21^{ras} and EF-Tu, in which the topologically corresponding regions are very much shorter (29 and 47 residues, respectively)[15]. Consequently it is reasonable to speculate that domain II may perform a function that is lacking in p21^{ras} and EF-Tu.

The high intrinsic rate of GTP hydrolysis by G proteins represents one such function. The signalling G proteins hydrolyse GTP at an unvarying intrinsic rate, whereas p21^{ras} and EF-Tu hydrolyse GTP at rates that crucially depend on the presence of other proteins. The k_{cat} for GTP hydrolysis by G proteins is relatively fast (>1 min^{-1}) and unaffected by binding to other proteins[21,22,27]. By contrast, p21^{ras} and EF-Tu hydrolyse GTP extremely slowly ($k_{cat\text{-}GTP}$ values of 0.02 min^{-1} or even lower[28,29]) unless their GTPase is stimulated by other proteins—GTPase-activating protein[9,10] in the case of p21^{ras} and the programmed ribosome in the case of EF-Tu (ref. 29).

Taken together, the parallel structural and functional differences raise the intriguing possibility that domain II serves as an intrinsic, 'built-in' GAP for G protein α-chains. This idea provides tentative explanations both for the large 'excess' sequence in domain II of G proteins relative to other GTP-binding proteins, and for the location of this excess sequence in α-chains; the topological equivalent of domain II in p21^{ras} contains the so-called 'effector' region (residues 33–40), in which mutational replacements of amino acids prevent interaction with GAP[10,30,31]. Thus the location of the postulated intrinsic GAP activity in the primary structure of G protein α-chains roughly coincides with the location of p21^{ras} residues which interact with a separate and distinct GAP protein; perhaps this coincidence implies similarly cognate locations of the two kinds of GAP in three dimensions as well. In this view, Arg 201 and cognate Arg residues in other G protein α-chains play key roles in the interaction between the intrinsic GAP-like domain and the GTP-binding domain. Mutations that replace Arg 201 or bacterial toxins that modify its side chain inhibit GTPase activity by interrupting this interaction. Although highly speculative, the postulated GAP-like function of domain II can be readily tested.

Is α_s an oncogene protein?

Are the mutant α_s proteins in group 2 pituitary tumours products of oncogenes? They resemble previously discovered dominantly acting oncogene proteins in four respects: (1) The mutant proteins mimic the effect of an extracellular growth factor—in this case, GHRH, which stimulates proliferation of normal GH-secreting pituitary cells by stimulating adenylyl cyclase[11]. (2) The mutations stabilize the active conformation of a signalling protein in a pathway that normally stimulates growth of the cell type carrying the putative oncogene. (3) The α_s mutations are found in tumour DNA but not in DNA from other cells (in this case, peripheral blood) of the same patients (Table 1). (4) The tumour genome contains both mutant and non-mutant

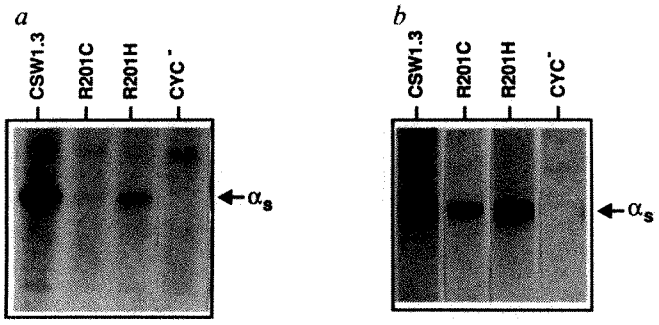

Fig. 4 Cholera toxin labelling and immunoblot analysis of wild-type and mutant α_s. *a*, The transfer of [^{32}P]NAD to α_s proteins by treatment of membranes (100 μg membrane protein) with cholera toxin was performed as described previously[21]. Labelled proteins were resolved on a 10% SDS polyacrylamide gel[21]. The labelling of R201X mutants, relative to labelling of the wild-type protein in csw1.3 membranes, was estimated by densitometric analysis of autoradiographs, using a LKB Ultrascan XL densitometer, and these values were corrected by the levels of α_s expression (see below). These calculations indicated that the toxin labelled R201X proteins much less efficiently than wild type α_s (<10% as well for R201C and 10–20% as well for R201H). *b*, To estimate the amount of wild-type or mutant α_s expressed in the *cyc*$^-$ clones, membrane proteins (90 μg csw1.3, R201C, and *cyc*$^-$; 70 μg R201H) were resolved on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose and immunoblotted[20,21] with affinity-purified antiserum directed against a peptide located in the carboxy-terminal half of α_s. Relative amounts of α_s were determined by densitometric analysis of autoradiographs and a standard curve constructed from blots of varying amounts of csw1.3 membranes (0–90 μg protein per lane). Estimates of these amounts, expressed as percentages of that in csw1.3 membranes (100%), were 34% in R201C (range = 31–38%, $n = 3$) and 115% in R201H (range = 89–149%, $n = 4$).

copies of the putative oncogene, indicating—as would be expected from the GTPase defect—that the effect of the mutant α_s is dominant with respect to wild-type α_s.

We have confirmed that a constitutively active α_s protein can produce dominant biochemical effects in tissue culture: expression of α_s-Q227L in Swiss-3T3 cells elevates cellular cAMP and augments DNA synthesis in serum-depleted medium containing insulin (I. Zachary, S.B.M. and H.R.B., manuscript in preparation). The straightforward implication—that the Q227 and R201 α_s mutations should produce similarly dominant effects on cAMP synthesis and proliferation of pituitary somatotrophs—must be qualified, however. Several findings hint that mutant α_s transcripts outnumber wild-type transcripts in group 2 tumours. Sequencing gels prepared from PCR-amplified genomic tumour DNA revealed wild-type and mutant bases at roughly equal intensities, indicating equal numbers of wild-type and mutant α_s genes. By contrast, direct sequencing of PCR-amplified cDNA from the same tumours invariably showed a more prominant signal from the mutant base (see Fig. 1). This is indicative of a preponderance of mutant α_s transcripts in group 2 tumours, which is corroborated by the disproportionately large number of mutant α_s cDNAs in M13 subclones (13 out of 15 sequenced; Table 1). Further investigation is required to determine whether expression of the mutant α_s genes in these tumours quantitatively exceeds that of wild-type α_s genes and, if so, to determine the mechanism responsible. At this stage we suggest that the biochemical dominance of GTPase-deficient α_s may be augmented by a second defect, so far not identified, that increases relative expression of the mutant α_s gene. Increased relative expression of dominant mutant oncogenes is often found in tumours, presumably because it confers a selective proliferative advantage on the tumour cell (for a summary, see ref. 32).

The most stringent criterion for qualification as an oncogene is that deliberate expression of the mutant gene should reproducibly induce formation of tumours. It is important to recognize that the postulated tumorigenic effect of mutant α_s would be exerted only in a relatively small subset of differentiated cells, those programmed to proliferate in response to elevated cAMP; this subset includes several endocrine target tissues (such as ovary, adrenal cortex, thyroid and some cells of the pituitary) and a small number of other cell types[1,2]. For this reason, stringent tests of the α_s oncogene hypothesis may require cell- or tissue-specific expression of mutant α_s genes in transgenic mice. In anticipation that such tests will be positive, we suggest that the postulated oncogene be designated *gsp*, for G_s protein.

Finally, it is worth noting that other G protein α-chains share with α_s a common mechanism for binding and hydrolysing GTP, as well as highly conserved primary structure in regions that correspond to the locations of Arg 201 and Gln 227 of α_s. Mutational replacement of these cognate residues in certain other α-chains will probably inhibit GTPase and generate constitutively active signals, such as those of the phosphoinositide/Ca^{2+} cascade. For this reason, we predict that new oncogenes will result from mutations in genes that encode the α-chains of G_i, G_o (refs 3, 4, 6, 33) and G_z (refs 34, 35). □

Received 28 June; accepted 18 July 1989.

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ACKNOWLEDGEMENTS. This work was supported in part by grants from the NIH and the March of Dimes, as well as the Italian National Research Council (special project 'Oncology'). We are grateful for the technical assistance of Dave Kabus, Susan Loughrey-Chen and Barry Beiderman, and the secretarial services of Helen Czerwonka.

Far-infrared observations of thermal dust emission from supernova 1987A

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INFRARED observations of supernova 1987A are important for studying both the evolution of the ejecta and its interaction with the surrounding interstellar medium. Here we report observtions of SN1987A, in the spectral range 18–35 μm, taken on 16 and 23 November 1988, 632 and 639 days after core collapse. A strong (~10 Jy at 20 μm) and rather flat continuum underlies weak fine-structure lines from heavy elements, and declines slowly between 24 and 30 μm. Its spectral shape is indicative of thermal emission from an almost featureless dust component, probably graphite, with silicates contributing <20% of the emitting dust mass. Some of the emission may be an 'echo' of supernova light reflected from a pre-existing dust cloud[1], but a better explanation, which can account for the entirety of emission from infrared to gamma wavelengths, is that dust is being formed in the supernova ejecta[2]. This also accounts more naturally for the inferred dust composition. Continuous observation is needed to determine the relative importance of these two components of the infrared emission.

Roche *et al.*[1] have found spatially extended emission at 10-μm wavelength which they attribute to a thermal infrared echo from a pre-existing dust cloud. If this cloud is responsible for the emissions we observe, we would also expect to see other effects, such as an inflection in the visual-light curve of the supernova, as the light from SN1987A is scattered off the dust cloud, as well as diffuse light around the supernova[3]. Both effects have been reported[3-6], but at a lower flux level than model calculations give, suggesting that only some fraction of the infrared light is an echo. At the same time, measurements of asymmetries in the O I and C I emission-line profiles[2] are evidence for the existence of some newly formed dust in the supernova ejecta.

The infrared observations of SN1987A were obtained on two flights with the Kuiper Airborne Observatory (KAO) on 16 and 23 November 1988. Details of the observations, the calibration, data reduction techniques and discussion of the fine-structure lines in our data will be presented in a separate paper (S.H.M., E.D., W.G., J.R.G., R.F.L. and R.F.S., manuscript in preparation). Here we concentrate on the continuum emission, shown in Fig. 1 together with selected data points from the 8–13 μm observations[1] that were obtained on 25 September (day 580 after core collapse). The spectrum shows the presence of weak fine-structure lines at 17.9 μm and at wavelengths >23 μm. The broad structure seen in the 19–23 μm region is still unidentified, and could be part of the underlying continuum emission. The continuum level is quite strong, ~10 Jy at 20 μm, and at the same level as the 8–13 μm observations on day 580. (The increase in the continuum at about day 450 (ref. 1) after a steady decrease from about day 120 and the steady decrease in the ionization state of the ejecta (ref. 1; S.H.M., E.D., W.G., J.R.G., R.F.L. and R.F.S., manuscript in preparation) suggest a thermal dust origin (instead of free-free radiation) for the emission. Furthermore, the smoothness of the spectrum limits the amount of silicate dust[3] (which has strong emission features at 9.7 and 18 μm) contributing to the emission.

Roche *et al.*[1] suggested that the emission is an infrared echo from a dust cloud located at a distance of about one light year behind the supernova, instead of dust that has recently formed in the expanding supernova ejecta. To calculate the mass and temperature of radiating dust, we constructed a simple dust model consisting of a population of graphite particles characterized by a single grain radius of 0.1 μm. We used graphite optical constants from ref. 7. Figure 1 shows the spectra of 300, 350 and 400 K graphite grains, normalized to produce the best visual fit to a lower envelope of continuum emission. The ambiguity in the dust temperature is due to the fact that the 8–13 μm data were not taken at the same time as the KAO observations were made, and the fluxes on day 635 could be higher or lower. A dust temperature of 400 K will require the 10-μm flux to be still rising on day 580, whereas a dust temperature of 300 K implies that it had already peaked before that time. At temperatures between 300–400 K the infrared spectrum peaks around 10 μm, and the relative flatness of the spectrum at longer wavelengths is a result of the enhanced emissivity of graphite (over that

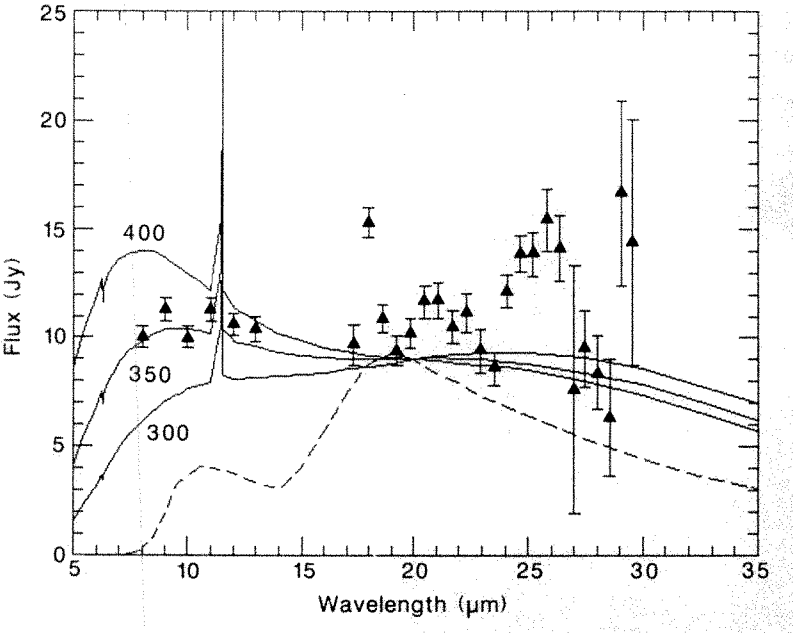

FIG. 1 The 18–35 μm spectrum of SN1987A obtained with the KAO on days 632 and 638 after core collapse, shown together with selected points from the 8–13 μm observations of Roche *et al.*[1]. The elevated points (triangles) in the 18–35 μm spectrum are due to emission from fine-structure lines of Fe I, S I and Fe II (S.H.M., E.D., W.G., J.R.G., R.F.L. and R.F.S., manuscript in preparation). The solid lines represents the infrared spectrum of a population of 0.1-μm graphite particles radiating at temperatures of 300, 350 and 400 K. Their spectrum was normalized to give the best visual fit to the underlying continuum emission in the 18–29 μm wavelength regime. Silicates of the same size and distance as the 400 K graphite dust will be heated to only ~180 K. Their spectrum (arbitrarily normalized to 9 Jy at 20 μm) is also shown (dashed line).

obtained by extrapolating the 10-μm value with a λ^{-1} emissivity law) in the ~20-μm wavelength regime. At 100 μm the infrared flux from these single-temperature dust models is <1 Jy. Our choice of graphite was motivated by the smoothness of the infrared spectrum. Amorphous carbon has a smooth spectrum as well, but it is not as broad as that of graphite, and the wide range of dust temperatures required to fit the observations with amorphous carbon might be hard to justify on theoretical grounds.

Some limits on the dust temperature may be set using the spatial extent of the emission[1] full-width half-maximum (FWHM) of 1.5 arcsec on day 580 and the echo delay time[3]. For dust temperatures of 300 and 350 K the model presented in ref. 3 shows that the projected radii of the radiating cloud will be 2.1 arcsec and 1.5 arcsec, respectively, which are larger than the reported FWHM. Adopting a dust temperature of 400 K, we calculate the total infrared luminosity and dust mass to be $5.9\times10^5\ L_\odot$ and $3.9\times10^{-4}\ M_\odot$, respectively. The derived distance, infrared luminosity and dust mass are similar to the values derived in refs 1 and 3, the main reasons for the differences being the more complete spectral coverage obtained with the additional KAO observations and the adopted dust model. The distance of the dust cloud is similar to that expected for the interaction distance between the winds of the red- and blue-supergiant progenitor[8,9], suggesting a circumstellar origin for the dust.

As discussed below, the infrared emission could also be due to dust that formed in the supernova ejecta. Roche *et al.*[1], however, advanced several arguments in favour of an echo interpretation for the emission: 1) scans across the remnant suggest that the emission is resolved, and the projected size of the source implies ejecta velocities of $\sim0.37c$, significantly larger than expected for any dust-forming ejecta; 2) the minimum size of the source cannot be smaller than that of a black body radiating energy at a rate $L_{IR}=5.9\times10^5\ L_\odot$ at a temperature of 400 K. The derived black-body radius on day 635, R_{BB}, is 1.2×10^{16} cm, corresponding to ejecta velocities of ~2,200 km s^{-1}. If the optical depth τ of the ejecta is about unity at 10 μm then for a λ^{-1} emissivity law, τ will be ≥20 at visual band wavelengths. The supernova should therefore be completely obscured in the visual band, contrary to observations (R. M. Catchpole *et al.*, preprint, 1988). One problem with a circumstellar origin for the dust cloud is its special location behind the supernova. Another problem is its implied carbon-rich composition[3]. Silicate particles, with the same radius of 0.1 μm, placed at the distance of the graphite dust cloud will attain a temperature of only 180 K. At this temperature the 18-μm silicate feature is very pronounced (Fig. 1). This feature and the one at 9.7 μm were suggested as spectral signatures of the composition of echoing dust shells around supernovae[10,11]. The 19–23 μm feature in our data could be considered as evidence for the presence of an underlying silicate feature. More detailed calculations show, however, that the feature needs to be narrower and slightly shifted to longer wavelengths to produce a good fit to the 19–23 μm 'bump'. We therefore estimate the contribution of silicates to be <20% of the total mass of dust giving rise to the infrared emission. This is contrary to the composition of dust expected to form in the oxygen-rich wind[9] of the red-giant progenitor. It may be that the emission is from interstellar dust around the supernova, that somehow was not cleared out of the ambient medium by the blue-supergiant wind.

A different origin for the infrared emission was suggested by Danziger *et al*[2], who reported optical evidence for the presence of condensed supernova dust in the expanding ejecta. During August–October 1988, the O I and C I emission lines became asymmetric with their peak emission blueshifted by 500–600 km s^{-1}. They attributed this development to extinction by dust that has formed in the metal-rich regions of the expanding ejecta, which may also be responsible for the 8–13 μm infrared emission. (There is a precedent for this phenomenon: line profiles in SN1979c became asymmetric about eight months after the explosion, with the peak emission blueshifted by ~700 km s^{-1}, a behaviour consistent with the appearance of dust in the supernova ejecta (R. A. Chevalier and R. F. Kirshner, personal communication).) The argument that SN1987A would be completely obscured by the dust[1] can be circumvented by adopting a model for the ejecta in which the dust resides primarily in clumps with $\tau\approx1$ and ≫1 at 10-μm and optical wavelengths, respectively. To comply with the black-body constraints described above, the clumps must subtend a projected area of at least πR_{BB}^2. To allow for a fraction of the supernova luminosity to escape the clumps need to be embedded within a larger volume of radius R, approximately given by the requirement that $N_c(R_c/R)^2<1$, where N_c and R_c are the total number of clumps and a typical clump radius, respectively. This gives $R>R_{BB}$. In this simple picture, the observed optical emission arises from the interclump medium with emission from the backside being more highly absorbed by the dust.

The attractive feature of the dust-formation model is that it offers a better explanation for the energy budget of the supernova during the epoch of the infrared emission. We consider the energetics of the supernova on day 613, for which the wavelength coverage is most complete. The total luminosity available on day 613 from the decay of an initial mass 0.078 $M_\odot$ of ^{56}Co is 4.87×10^{39} erg s^{-1} (R. M. Catchpole *et al.*, preprint, 1988). The luminosity observed in the U–M bands is $1.38(\pm0.16)\times10^{39}$ erg s^{-1}, the errors reflecting uncertainties in the integration over wavelengths. Various models predicting the X- and γ-ray luminosity L_γ from SN1987A were summarized by Catchpole *et al.* (preprint, 1988). Their predictions for L_γ on day 613 are in the range $(1.5\text{–}2.5)\times10^{39}$ erg s^{-1}. Of these models, the γ-ray observations on day 433 (ref. 12; N. Gehrels, personal communication) are more consistent with the model of Pinto and Woosley[13], which predicts $L_\gamma=2\times10^{39}$ erg s^{-1} on day 613. The analysis of the γ-ray data obtained on that day is still in progress (N. Gehrels, personal communication). We will therefore adopt here a value of $2.0(\pm0.5)\times10^{39}$ erg s^{-1} for L_γ on day 613, with the error reflecting the range of model predictions. We estimate the infrared luminosity for day 613 to be $1.7(\pm0.7)\times10^{39}$ erg s^{-1}. The error in this case reflects the uncertainties in their luminosities for days 580 and 635. Summing up, the total bolometric luminosity from the supernova is $5.1(\pm1.4)\times10^{39}$ erg s^{-1}, consistent with the available cobalt decay energy. In the echo model, the infrared and part of the visible light is merely reprocessed light or a reflection from the dust cloud[3], and should therefore not be included in the bolometric supernova output. The total bolometric luminosity of the supernova would then be $<3.4(\pm0.7)\times10^{39}$ erg s^{-1}, which accounts for only ~70% of the cobalt decay energy.

If the emission originates from newly formed dust then the infrared observations may constitute the 'missing link' between laboratory data of isotope composition anomalies in meteorites and theories on their origin. The dust-formation model does not constrain the composition of the dust in the ejecta, which could be either carbonaceous, silicate or even both, because neither C nor O in the ejecta will be completely locked in CO molecules[14]. The formation of carbonaceous dust in SN1987A, however, could explain the identification by Clayton[15] of the He-rich layers of supernovae as a site for the production of anomalously heavy Xe (formed by the intense neutrino burst). With some of the Xe locked up in carbon grains, this accounts for the presence of these isotopic composition anomalies in meteoritic diamonds[16].

Continuous infrared observations are extremely important to determine the origin of the infrared emission. In the dust-formation model, the emission will remain spatially unresolved, and its spectral behaviour is determined by the available energy sources in the ejecta at the time of the observations[17]. Consequently, we expect the dust temperature to continuously decrease because of the Co decay and the increasing trans-

parency of the ejecta. With $\sim 10^{38}$ erg s^{-1} as a recently defined upper limit on the luminosity of the underlying pulsar[4], we do not expect any future rise in dust temperature. In the echo model, the dust temperature decreases due to the combined effects of the Co decay, and the geometry of the dust cloud. Depending on the cloud geometry, we may soon observe spatial as well a spectral evolution in the infrared emission. □

Received 24 March; accepted 7 July 1989.

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ACKNOWLEDGEMENTS. We thank the crew and staff of the KAO for their support during the observations. We thank C. Scearce, C. Lowe, C. Sappington and D. Dargo for their help in the preparation of the instrument, and K. Stewart and J. Heaney for their assistance in its calibration. We thank J. Felten for discussions. This work was supported by the NASA Airborne Astronomy Program.

Pulsational magnetic radiation from the neutron star in supernova 1987A

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THE 0.5-millisecond period of pulsar PSR0535-69 in supernova 1987A (ref. 1) may be hard to explain by a conventional rotating neutron-star model, but might more naturally be understood if the neutron star is undergoing radial pulsation[2]. If this is the case, the pulsations will give rise to a time-varying dipole moment, and thus to electromagnetic radiation. I show here that any pulsar heating of the supernova remnant greater than $\sim 1.5\times 10^{37}$ erg s^{-1} must be due to this pulsational magnetic radiation (PMR). Recent observations[3,4] suggest a pulsar power $\sim 7\times 10^{37}$ erg s^{-1}. This implies that PMR is indeed heating the supernova nebula, and that the amplitude of the neutron-star surface oscillation after ~ 2 yr is $\sim 10\, B_{12}^{-1}$ m, where B_{12} is the polar magnetic field in units of 10^{12} Gauss. The vibrating pulsar model makes several testable predictions: in particular, any PMR component in the light curve will have a characteristic decay time of ~ 0.5 yr (with large uncertainty) which is the result of rotation-induced damping by gravitational radiation. This would be very different from the usual pulsar heating rate, which is approximately flat on timescales of the order of years.

If the 0.5-ms pulses are due to rotation, then bounds on the supernova-remnant energy excess imply a polar field strength $B \leq 10^9$ G (ref. 1). If, however, the pulses are due to radial oscillations excited at the neutron star's birth, then the requirement that rotation-dependent gravitational radiation does not damp the oscillations too quickly[2] implies $P_{\rm rot} > 0.1$ s, and other damping limits described below imply $B < 10^{13}$ G. Comparing these two candidate models with the Crab pulsar ($P_{\rm Crab} \approx 20$ ms at birth[5], $B_{\rm Crab} \approx 5\times 10^{12}$ G), we find that the pulsating model star has ≥ 5 times the rotation period and essentially the same magnetic field as the Crab, whereas a 0.5-ms rotator has 1/40th the period and $\leq 2\times 10^{-4}$ the field strength. Because the Crab parameters are typical for observed young pulsars[5], including those in the Large Magellenic Cloud[6], here I shall concentrate on the pulsation model.

The energy of the fundamental radial mode of an oscillating neutron star is related to the linear oscillation amplitude $\delta \equiv \Delta R/R$ by

$$E = 3\times 10^{53}\, \delta^2 M_s^2 R_{12}^{-1} \quad \text{erg} \tag{1}$$

where $M_s \equiv M/M_\odot$ and $R_{12} \equiv R/12$ km. This formula is a fit to calculations[7] which employ the Bethe-Johnson equation of state for nuclear matter. The corresponding masses and radii are from ref. 8. The Bethe-Johnson equation of state is in the mid-range of hardness among current candidates[5]. Because of the M, R scalings, equation (1) is correct to within a factor of roughly two for the other equations of state considered in ref. 7, although it breaks down for neutron stars close to their limiting masses.

Because magnetic flux cannot migrate through the crust on the timescale of a pulsation period, the magnetic-field variations are confined to the surface and so vary as R^{-2} during pulsations. This leads to a time-varying dipole moment,

$$|\mathbf{M}| = \frac{BR^3}{2} \propto R(t) \tag{2}$$

where B is the field strength at the magnetic pole. The resulting PMR has power

$$\dot{E}_{\rm PMR} = \frac{B^2 R^6 \omega_{\rm pls}^4 \delta^2}{12c^3} \tag{3}$$

where $\omega_{\rm pls}$ is the pulsation angular frequency $2\pi/P_{\rm pls}$. This mode of neutron-star emission has been independently suggested by a number of authors[9-11].

A comparison of equations (1) and (3) shows that PMR damps the pulsation energy on an folding timescale (defined as $-E/\dot{E}$)

$$\tau_{\rm PMR} \approx 40\, B_{12}^{-2} R_{12}^{-7} M_s^2 \quad \text{yr} \tag{4}$$

The condition that PMR does not damp out the oscillations before the time at which they were observed implies

$$B \leq 10^{13}\, R_{12}^{-7/2} M_s \quad \text{G} \tag{5}$$

Together with the gravitational-wave damping limit $P_{\rm rot} > 0.1\, R_{12}^{1/2} M_s^{1/4}$ s mentioned above[2], this implies that the rotational magnetic-dipole radiation, RMR (that is, the component of emitted power peaked near $\omega_{\rm rot} \equiv 2\pi/P_{\rm rot}$), is

$$\dot{E}_{\rm RMR} < 1.4\times 10^{37}\, R_{12}^{-3} M_s \quad \text{erg s}^{-1} \tag{6}$$

A more rigorous argument confirms this result[12].

There is tentative evidence that a central pulsar is energizing the SN1987A remnant at a rate (on day ~ 750) just slightly below $\sim 10^{38}$ erg s^{-1}. On 1989 March 6 it was announced[3] that the bolometric light curve exhibits a steady component of 7×10^{37} erg s^{-1}. When this component is subtracted from the data, a constant exponential decline is found from day 520 to 730 (ref. 3). Furthermore, soft X-rays (6-10 keV) observed by the Ginga satellite have been interpreted as synchrotron emission from a central non-thermal nebula powered at the $\sim 10^{38}$ level[4,13]. If remnant heating in this range is verified by future studies, then equation (6) implies that the low-frequency RMR is not adequate to power the nebula and one must conclude that kHz-frequency pulsational magnetic radiation is responsible for heating the SN1987A nebula.

In a PMR-heated supernova, the amplitude of the radial pulsations can be estimated using equation (3):

$$\delta = 6\times 10^{-4}\, B_{12}^{-1} R_{12}^{-3} L_7^{1/2} \tag{7}$$

where L_7 is the pulsar power in units of 7×10^{37} erg s^{-1}. For homologous motion (which is a reasonably accurate assumption[7]) and $L_7 = 1$ this corresponds to a degenerate surface oscillation of $\Delta R \approx 14\, B_{12}^{-1} R_{12}^{-2}$ m, peak to peak. Adopting the upper

limit on B from equation (5), which agrees with empirical properties of the pulsar population[5], I obtain a lower limit on the amplitude $\delta > 6\times10^{-5}\,L_7^{1/2}$ or (roughly) $\Delta R > 1.4$ m. Of course, plasma effects close to the pulsar (for which there is evidence in SN1987A, as mentioned above[4,13]) complicate the idealized emission mechanism described here and might change these results by factors of the order of unity[5].

Although PMR heats the nebula, rotation-induced gravitational radiation is the dominant pulsation-damping mechanism (unless either $P_{\rm rot} > 0.3\,B_{12}^{1/2}$ s or neutrino damping is enhanced by some exotic interior composition[2]) with a timescale

$$\tau_{\rm GR} \approx 0.5\left(\frac{P_{\rm rot}}{0.1\ {\rm s}}\right)^4 \ {\rm yr} \qquad (8)$$

The only detailed, published calculation[14] of $\tau_{\rm GR}$ is based on uniformly rotating constant-density model stars undergoing homologous radial oscillations. (Reference 15 contains an earlier estimate.) Real neutron stars are centrally condensed by a factor of roughly four or more, depending on the uncertain equation of state at supranuclear densities[8] and a crude estimate (L. Jin, personal communication) shows that this decreases the efficiency of gravitational-wave emission by a factor of roughly two from the value in ref. 14. This factor of two has been included in the above estimate.

As an illustrative calculation, I express the theoretical uncertainty in $\tau_{\rm GR}$ in terms of a parameter η. The initial amplitude of oscillations of the neutron star becomes

$$\delta_0 \approx \delta\,\exp\left[2\eta^{-1}\left(\frac{P_{\rm rot}}{0.1\ {\rm s}}\right)^{-4}\right] \qquad (9)$$

where δ is the present amplitude (~day 700). Using $P_{\rm rot} = 0.1$ s and $\eta = 1$, the initial amplitude was $\delta_0 \approx 0.01\ (\delta/10^{-3})$. This implies that the nascent neutron star had a radial oscillation energy $E_0 \approx 3\times10^{49}\,\delta_3^2$ ergs, where $\delta_3 \equiv \delta/10^{-3}$. This energy was presumably left over after the star's core bounce was damped by driving the supernova shock; the fundamental radial mode might be excited with comparable strength in all nascent neutron stars. Most of the pulsational energy was lost to gravitational radiation. The total energy imparted to the supernova nebula by PMR since birth of the neutron star is

$$E_{\rm neb} = \frac{E_0\tau_{\rm GR}}{\tau_{\rm PMR}} \approx 10^{-2}\,E_0\left(\frac{P_{\rm rot}}{0.1\ {\rm s}}\right)^4 B_{12}^{-2} \qquad (10)$$

In the case considered above, $E_{\rm neb} \approx 3\times10^{47}\,\delta_3^2$ erg. The greatest share of this was emitted in the first $\tau_{\rm GR}$ and increased the supernova explosion energy by only ~3 parts in 10^4 for $\delta_3 = 1$. Note that $\tau_{\rm GR}$ is $\sim 3\eta$ times the present decay time of the radioactive ^{56}Co component of the light curve (which has been gradually diminishing because of increasing transparency to γ-rays[3,16]) so it is understandable that the PMR component of the light curve became evident only after ~2 yr.

Attempts by the original observers[1] and others [17] to reobserve the 0.5-ms pulses have failed. This fact, along with the change in the pulse luminosity during the original observing run, have led to speculation that variable dust obscuration has been present[1]. The apparent blueshifts of emission lines give evidence for recent dust formation in the supernova nebula[18]. Furthermore, observations of the SN1987A infrared excess made with the Kuiper Airborne Observatory suggest that clumpy dust has formed[19]. Calculations show that temperatures could reach the dust condensation temperature in the metal-rich mantle as early as day ~600 (ref. 20).

If the neutron star is truly obscured, it still might be possible to corroborate the pulsation model because it makes very distinctive predictions for the supernova light curve. (Of course, the effect of dust on the light curve itself must be taken into account.) After radioactive heating drops below $\dot{E}_{\rm PMR}$ at a crossover time t_1, the bolometric luminosity L enters a phase of more gentle exponential decline:

$$L(t) = L_1\,\exp\left(-\frac{t-t_1}{\tau_1}\right) \qquad (11)$$

where

$$\tau_1 \equiv (\tau_{\rm GR}^{-1} + \tau_{\rm PMR}^{-1} + \tau_\nu^{-1})^{-1} \qquad (12)$$

In this equation τ_ν is the neutrino damping time, which is $\sim 10^2$ yr unless some exotic enhancement occurs[2,21] so this term is probably not important. Furthermore, if $P_{\rm rot} < 0.3\,B_{12}^{-1/2}$ s, as seems likely, then $\tau_1 \approx \tau_{\rm GR}$.

The light curve should therefore show a $\tau_{\rm GR} \sim 200$-day slope to the log L (pulsar) component. This is very different from the flat (on a timescale of years) contribution of rotation-driven pulsar heating.

This circumstance underscores the need for diligent monitoring of the light curve during the next two years or so. Of course, the precise value of $\tau_{\rm GR}$ is difficult to predict because of its steep dependence on $P_{\rm rot}$ and present theoretical uncertainties (compare equation (8)).

The luminosity phase of equation (11) persists until $L(t)$ reaches a value at time t_r

$$L_2 \approx 10^{35}\,B_{12}^2\left(\frac{P_{\rm rot}}{0.1\ {\rm s}}\right)^{-4} R_{12}^6 \quad {\rm erg\ s^{-1}} \qquad (13)$$

at which time the low-frequency rotational luminosity becomes important and the light curve asymptotically reaches

$$L(t) = \frac{L_2\tau_2^2}{[\tau_2 + (t-t_2)]^2} \qquad (14)$$

where τ_2 is the energy decay rate due to spindown at time t_2:

$$\tau_2 \equiv \frac{1}{2}\,I\omega_2^2 L_2^{-1} = 10^5\,B_{12}^{-2} I_{45}\left(\frac{P_{\rm rot}}{0.1\ {\rm s}}\right)^2 \ {\rm yr} \qquad (15)$$

where I_{45} is the moment of inertia in units of 10^{45} g cm^2 and ω_2 is the rotational angular frequency at time t_2. The decline in this final phase is indetectably small, although the associated $\dot{P} = P_{\rm rot}/\tau_2 \approx 10^{-14}$ could be detectable if the pulsar is detected at radio frequencies.

Measuring the decay time of the pulsar contribution to the light curve at $t < t_2$ will determine $P_{\rm rot}$. If the levelling off at L_2 is detected (which may require waiting until the contribution from long-lived isotopes like ^{57}Co decreases as a result of decreasing γ-ray absorption[13]) this will determine B. In this way one might deduce the amplitude of the initial oscillations through equations (7) and (9) without seeing the central star again. If a radio pulsar is found, $P_{\rm rot}$ will be directly measured and B determined from $\dot{P}$, thus the pulsation model could conceivably be confirmed without any second observation of the 0.5-ms pulses. □

Received 23 March; accepted 13 July 1989.

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ACKNOWLEDGEMENTS. I thank L. Jin, P. Shapiro, C. Thompson, E. Vishniac and C. Wheeler for discussions. This research was supported by the Texas Advanced Research Program.

The acceleration of pulsars—a new test

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If pulsars are accelerated by the emission of asymmetric magnetic dipole[1] or neutrino[2] radiation, the spatial velocity acquired should lie along its axis of rotation. A simple test[3] of such models is that the position angle on the sky of the proper motion φ_v and the polarization plane at the peak of the pulse profile φ_p should be either parallel or perpendicular. This hypothesis does not appear to be borne out by the available observations[4]. We have proposed[5] a more direct 'scalar' test of the acceleration idea: the tangential velocity V_t of a pulsar (that is, the projection of its actual spatial velocity) should be proportional to the sine of the angle ζ between the line of sight and the rotation axis.

Table 1 presents data for 29 pulsars with well-known tangential velocities[6–10] and known distances[8,11]. We used estimates of the angles β between the magnetic and rotational axes as well as the values $\zeta = \beta + |\zeta - \beta|$ and $|\zeta - \beta|$ for 21 pulsars from ref. 12. Average values of ζ were used for all cases except that of PSR 0833-45 for which the smaller value was used. The statistical method for the determination of these angles[12] uses the maximum rate of change of the internal position angle of the polarization plane and the value of the cone semiangle of the pulsar radiation, as in ref. 13. However, the angles θ for the 400-MHz frequency in ref. 12 are 1.42 times larger than in ref. 13 and the values of the angles β and ζ are therefore systematically greater.

Using reduction formulae we obtained estimates of ζ and $|\zeta - \beta|$ for PSR0329+54, 0736+40, 1541+09 and 2020+28 from the data in ref. 13. For PSR0355+54 and 1604−00 we obtained estimates of ζ in the same manner as in ref. 13.

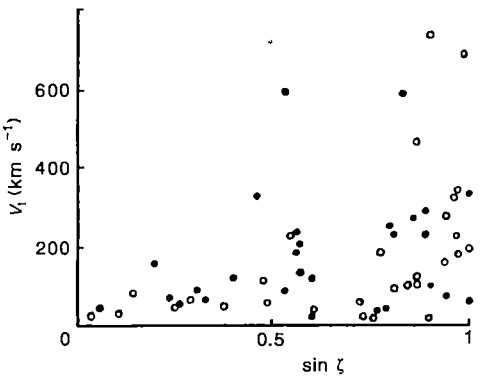

FIG. 1 Tangential velocities of pulsars as a function of sin ζ. Open circles refer to pulsars in Table 1. The filled circles refer to 28 other pulsars for which ζ and V_s, the velocity of the interstellar-medium producing scintillation of the pulsars, are known[11]. The values of V_s are, on average, smaller than V_t by a factor of 2.1.

We used position angles φ_p of the polarization planes at the centres of the pulse profiles of radio emission from refs 14–16, where possible, we used recent estimates of the Faraday-rotation measure[17]. Where the angle of the plane of polarization inside the pulse profile changed abruptly, the angle $\varepsilon = |\varphi_p - \varphi_v|$ increased by 90° as reported in ref. 14 except for the case of PSR0355+54; these cases are marked by asterisks. The last column of the table gives the estimates of the spatial velocities, $V = V_t/\sin\zeta$.

Figure 1 shows the correlation of V_t and sin ζ. The model of the acceleration of the pulsar along the rotation axis and the model of its radiation through the polar cap[18], heretofore unrelated, are mutually justified.

The mean spatial velocity of the pulsars referred to in Table 1 is 257±37 km s^{-1}, for the additional 28 pulsars $\bar{V}$ is 308±47 km s^{-1}, and for the whole set $\bar{V}$ is 282±38 km s^{-1}. The

TABLE 1 Kinematic data of pulsars

PSR	Distance (kpc)	V_t (km s^{-1})	$\lvert\zeta\rvert$ (deg.)	$\lvert\beta-\zeta\rvert$ (deg.)	φ_p (deg.)	φ_v (deg.)	ε (deg.)	V (km s^{-1})
0301+19	0.56	101±14	57	3.4	23±2	−9±11	32±11	121±16
0329+54	2.30	225±25	33	2.9	30±13	−56±2	86±13	412±46
0355+54	1.64	66±31	17	1.5	138±2	41±27	83±27	226±106
0531+21	2.00	157±41	69	16.0	140±1	−64±6	24±6	168±44
0611+22	3.07	131±57	60	9.6	16±1	0±43	16±43	151±66
0736−40	2.00	684±80	80	7.3	—	−50±8	—	694±81
0809+74	0.18	43±6	14	3.8	152±5	163±8	79±9*	178±25
0823+26	0.36	193±19	88	3.1	10±3	−33±1	83±3	193±19
0833−45	0.50	36±13	37	14.5	63±2	−126±16	9±15	60±22
0834+06	0.43	105±16	60	4.3	123±9	2±6	59±11	121±18
0943+10	0.56	115±59	28	6.8	—	−119±17	—	245±126
0950+08	0.13	25±3	6	6.8	172±6	27±6	35±8	239±29
1133+16	0.16	278±28	70	6.0	117±7	−12±1	51±7	296±30
1237+25	0.33	178±18	76	2.3	76±4	−68±1	36±4	183±18
1508+55	0.73	346±36	76	5.8	8±4	−133±2	39±4	357±46
1541+09	1.34	81±31	8	1.0	45±12	−75±14	60±18	583±223
1604−00	0.36	14±15	63	14.4	175±10	−174±22	11±24	16±19
1642−03	0.16	20±8	47	0.8	65±15	168±12	77±19	87±11
1818−04	1.50	187±25	51	3.9	79±2	6±7	73±2	241±32
1929+10	0.11	55±6	29	8.1	65±5	64±3	1±6	113±6
1933+16	6.00	733±130	64	12.5	167±9	175±8	8±12	816±145
1944+17	0.47	20±9	2	1.7	101±9	−6±32	73±2	571±257
1952+29	0.21	44±11	22	5.8	—	145±20	—	117±29
2016+28	1.30	15±13	49	6.4	94±2	57±24	53±24*	20±17
2020+28	1.30	97±15	54	3.0	179±10	35±10	36±14	120±18
2021+51	0.70	59±13	46	7.6	29±2	19±13	10±13	82±18
2045−16	0.39	465±86	60	1.7	91±21	7±3	84±21	537±100
2217+47	1.46	225±47	76	6.3	81±6	22±14	59±15	232±48
2303+30	1.91	321±63	73	7.0	—	159±13	—	336±66

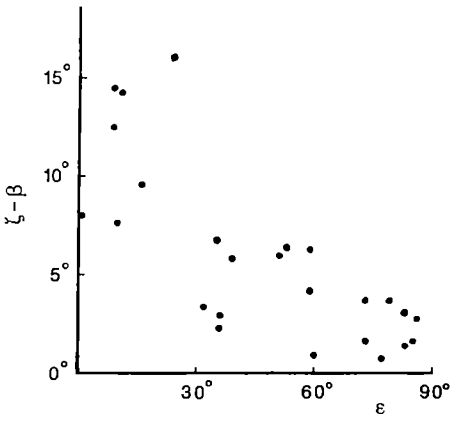

FIG. 2 $|\zeta-\beta|$ as a function of ε.

exclusion, in both samples, of three pulsars having small ζ-values gives $\bar{V}=233\pm38\,\mathrm{km\,s^{-1}}$ for 26 pulsars from Table 1, $\bar{V}=246\pm29\,\mathrm{km\,s^{-1}}$ for 25 pulsars from additional sample and $\bar{V}=224\pm29\,\mathrm{km\,s^{-1}}$ for the whole set. Spatial velocities $>500\,\mathrm{km\,s^{-1}}$ are obtained for five of the pulsars mentioned in the table and for five of the pulsars from the additional set. In both of these sets two pulsars have small ζ-values of less than 11° and therefore their values of V are unreliable. Three pulsars in the additional set have anomalously large values of the interstellar-medium velocity V_s and the distances of PSR0736−40 and 1933+16 have probably been overestimated. Therefore the only pulsar for which there are no suspicions about the high velocity V is PSR2045−16 and so we believe that it would be premature to postulate the existence of a secondary maximum in the velocity distribution above $500\,\mathrm{km\,s^{-1}}$.

The data in the table indicate a uniform ε-distribution, which is in agreement with a previous study[4]. The postulate[3] that the values of ε may be only 0° and 90° is incorrect. Figure 2 shows the relationship between ε and $|\zeta-\beta|$, which may be attributed to the change in ζ for different intersections of the pulsar radiation cone with the line of sight. Therefore, the value of ε changes monotonically from 0° for $|\zeta-\beta|\approx\theta$, to 90° for $|\zeta-\beta|\approx0°$. □

Received 13 February; accepted 20 June 1989.

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Submarine venting of phase-separated hydrothermal fluids at Axial Volcano, Juan de Fuca Ridge

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SINCE the discovery of high-temperature venting on the East Pacific Rise in 1979[1], it has been expected[2,3], because of the physical properties of sea water at pressures and temperatures encountered during submarine hydrothermal circulation[4,5], that phase-separated fluids would discharge from ridgecrest vents. Although this notion is supported by the reported large deviations in vent-fluid chlorinity relative to that of sea water (−40%–+200%)[6,7], by observations of venting at *P–T* conditions clearly within the two-phase region (220 bar and 420 °C)[8] and by fluid-inclusion data[9,10], unequivocal identification[11] of phase-separated venting fluids has remained elusive. Here we report observations of chloride- and metal-depleted, gas-enriched fluids from a shallow vent field on the Juan de Fuca Ridge which confirm the expectation that phase-separated effluents are delivered to the deep ocean from some sea-floor venting systems.

The ASHES (Axial Seamount Hydrothermal Emissions Study) vent field lies at a depth of 1,542 m within the caldera of Axial Volcano on the Juan de Fuca Ridge (Fig. 1) and is the surface expression of the shallowest active high-temperature sea-floor venting system known[12]. Within a 60-m-diameter area located at the northern end of ASHES, four sulphide edifices (Inferno, Hell, Hillock and Mushroom, Fig. 1) rise 1–4 m above bare lobate basalt and vent clear, brown and black fluids at temperatures reaching 328 °C. Clear, high-temperature fluids also discharge from two unusual vent structures located within this region: Virgin Mound ($T_{max}=299$ °C), a <0.7-m tall, white anhydrite chimney that rebuilds <24 h after demolition, and Crack Vent ($T_{max}=219$ °C), a several metre long, 7- to 10-cm wide, anhydrite-filled fracture in sheet-flow basalt. Low-temperature ($T_{max}=55$ °C) fluids issue from smaller cracks located above the vent field.

Vent-fluid samples were collected at the ASHES site in July 1986 using the research submersible *Pisces IV*[12], and in September 1987 and August 1988 with DSV *Alvin*. The results for selected vent-fluid properties are shown in Fig. 2 and the hydrothermal endmember compositions, estimated by extrapolation of the entire data set to zero Mg concentration[13], are given in Table 1. The low Mg values observed and the reproducible trends of the ASHES data throughout the two-year sampling period (Fig. 2) support the use of the above method for estimating endmembers.

Because Cl^- is the major anion in vent fluids and because chlorinity serves as an approximate measure of the total dissolved salts, we have arranged the vent-fluid data into three groups based on endmember Cl concentrations relative to the ambient seawater value of $539\,\mathrm{mmol\,kg^{-1}}$ (Fig. 2, Table 1). Inferno is the only vent sampled with a Cl concentration significantly greater than that of sea water, $625\,\mathrm{mmol\,kg^{-1}}$; data from Inferno is referred to as Cl-enriched. Hell, Hillock, and Mushroom vent fluids have endmember Cl concentrations in the range 480–$540\,\mathrm{mmol\,kg^{-1}}$ (composite value $515\,\mathrm{mmol\,kg^{-1}}$) and are here termed Cl-normal. Cl-depleted fluids were collected from Virgin Mound, Crack and low-temperature vents and for these the endmember Cl concentrations are in the range 176–$258\,\mathrm{mmol\,kg^{-1}}$ (composite value $188\,\mathrm{mmol\,kg^{-1}}$). The Cl-depleted fluids are also notably depleted in metals and enriched

in gases. The observation of such large variations in chlorinity within a single vent field, with different vent types sometimes located within only a few metres of one another, is unprecedented.

The abnormally low Cl and metal and high gas concentrations of the Cl-depleted fluids are the most remarkable attributes of the ASHES data. The Cl-endmember concentration is 35% of the Cl concentration of sea water and is by far the lowest reported value for ridgecrest fluids. Rock hydration and variable precipitation/dissolution of an unidentified chloride-bearing mineral, which have been proposed to explain Cl concentrations differing from that of sea water at other ridgecrest systems, are not adequate to explain the magnitude of the ASHES Cl depletion[6,7,13–15]. Endmember values of Fe, Zn and Mn are only 1%, 2% and 16% of the respective Cl-normal values and are much lower than concentrations reported for other bare basalt high-temperature venting sites[6,7,14,16]. The higher pH, cooler temperature and lower Cl concentration of the vapour-enriched fluids probably all contribute to the low metal loadings[6,17]. The total condensable-gas concentration of the Cl-enriched fluid (50 mmol kg^{-1}, assumed to be CO_2 because H_2S was removed before analysis) is similar to that predicted from an earlier measurement of warm spring discharge at the Canadian–American Seamount (CASM) site[18] (Fig. 1) and is an order of magnitude higher than values reported for fluids from other unsedimented ridgecrests[6,19]. Even more striking and significant is that the gas concentration (285 mmol kg^{-1}) of the Cl-depleted endmember is almost six times higher than that of the Cl-enriched endmember.

We believe that the unusual characteristics of the Cl-depleted endmember can be best explained by phase separation, phase segregation[20] (that is, unmixing, to various degrees, of the vapour and liquid phases) and, finally, addition of a small amount of cold sea water to the vapour-enriched mixture. We know of no other mechanism capable of producing the large deficits of Cl, Br and Na in the Cl-depleted fluids. The fact that the concentrations of Li and K, species which are conservative during seawater mixing, from all of the high-temperature vents at ASHES can be described (after removal of the seawater component of the mixtures) by a single mixing line having a freshwater-like fluid as its origin (Fig. 3), is strong support for the hypothesis that phase separation and segregation are important processes that control venting chemistry at this site.

Maximum venting temperatures of 295 °C, 298 °C and 299 °C were measured at Virgin Mound during 1986, 1987 and 1988, respectively. For Inferno vent, the corresponding maximum temperatures were 328 °C, 326 °C and 319 °C. Although the gas concentration at Inferno is sufficient to lower the sea-floor boiling temperature by 3–5 °C (ref. 21), all of the measured temperatures are at least 20 °C below the sea-floor boiling temperature (348 °C (ref. 22)), indicating that some combination of conductive heat transfer to host rock and/or mixing with cold sea water has taken place. As heat loss accompanies boiling and because pressures are greater in the subsurface, initial boiling temperatures must have been significantly greater than 350 °C.

The relative gas concentrations of the Cl-enriched and Cl-depleted endmembers provide critical support for the phase-separation hypothesis[6,11]. By extrapolating gas solubility data for sub-critically boiling salt solutions beyond 350 °C (ref. 17), a fully segregated vapour formed by boiling sea water at, for example, 370 °C should be ten times more concentrated in CO_2 and five times more concentrated in H_2S, relative to the residual liquid phase. Gas partitioning to the vapour phase would be reduced for boiling at higher temperatures. Although uncertainty

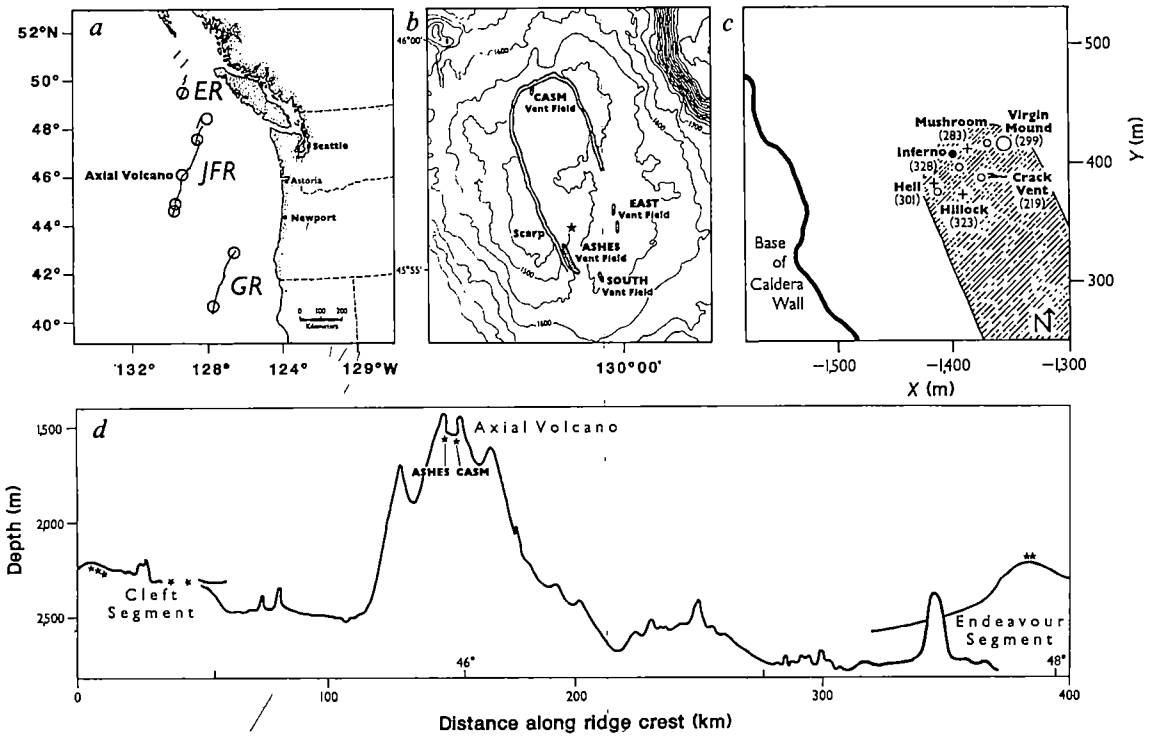

FIG. 1 *a*, Location map of Axial Volcano on Juan de Fuca Ridge (JFR), north-east Pacific Ocean. Explorer Ridge (ER) and Gorda Ridge (GR) also shown. Circles mark known venting sites. *b*, Position of ASHES vent field within caldera of Axial Volcano: ★ tied to high-temperature venting region that is confined to northern end of vent field. CASM, East and South vent fields support low-temperature ($T \leq 100$ °C) venting[18,28]. Metal-sulphide- and barium-enriched chimneys, evidence for past high-temperature discharge, have been recovered from CASM and East sites. Contour interval of bathymetry, 50 m. *c*, Detail map of high-temperature venting domain within ASHES vent field sampled in 1986, 1987 and 1988. High-temperature vents: ● and +, 1–4 m high sulphide edifices with multiple orifices; ○ and ⌐, <0.7-m-high anhydrite chimney and several metre-long, 7–10 cm wide anhydrite-filled cracks. Maximum observed venting temperatures given in parentheses (°C). Low-temperature ($T \leq 55$ °C) vent sampling sites, ○. Vent structures sit on unsedimented lobate and sheet-flow basalt at depth of 1,542±1 m. Coordinates are relative to acoustic navigation net ($x-1375$, $y+400$ equivalent to GPS position: 45°56.0′ N, 130°00.9′ W). *d*, Juan de Fuca ridgecrest bathymetry showing relatively shallow depth of ASHES vent field. Stars mark locations of known vent fields.

TABLE 1 Endmember values for ASHES vent fluids and regional sea water

	Cl-enriched	Cl-normal	Cl-depleted	Sea water
Temperature (°C)	149–328	136–323	5–299	2.42
pH (NBS, 25 °C)	3.5	3.5	4.4	7.47
Alkalinity (μeq kg^{-1})	−453	−519	580	2,354
Condensable gases (cm^3 STP g^{-1})	1.1	ND	6.3	0.05
H_2S (mmol kg^{-1})	7.0 (7.5)	8.1	(19.5)	0
$Si(OH)_4$ (mmol kg^{-1})	14.7	15.8	13.8	0.169
Mg (mmol kg^{-1})	0	0	0	52.4
Cl (" ")	625	515	188	539
Na (" ")	500	415	159	462
Ca (" ")	46.8	37.3	10.2	10.3
K (" ")	27.5	22.0	7.6	9.8
Br (" ")	0.95	0.76	0.24	0.83
Li (" ")	0.637	0.512	0.204	0.026
Mn (μmol kg^{-1})	1,133	1,016	162	0.001
Fe (" ")	1,071	925	9	0.009
Cu (" ")	12	10	0.7	0.004
Zn (" ")	114	115	2.3	0.009
Pb (" ")	0.34	0.30	0.10	0.00001
P (" ")	0.09	0.30	0.31	3.07
Ge (" ")	0.145	0.148	0.126	0.000122
B (" ")	565		503	421
$\delta^{18}O$ (‰)	+0.8–+1.1		+0.7–+0.9	−0.2

Water samples were collected in 755-ml titanium-piston syringes[14]; total condensable-gas samples in 150-ml titanium gas-tight bottles (supplied by J. Lupton). In 1987 and 1988 titanium sample reservoirs were coupled to an insulated manifold sampling system equipped with a platinum-resistance thermometer (precision: ±1 °C) at the intake nozzle; range of collection temperatures given. pH (precision: 0.3%) and alkalinity (0.2%) determined potentiometrically. Total condensable gases (3%) determined manometrically; H_2S removed by precipitation as HgS before determination. H_2S (5%), $Si(OH)_4$ (1%), and P (2%) determined by flow-injection visible spectrophotometry; Mg (0.5%) and Ca (0.5%) by EDTA–EGTA titration; C by potentiometric titration (0.2%); Br (5%) by visible spectrophotometry; K (4%), Li (2%), Mn (2%), Fe (7%) and Zn (2%) determined by flame atomic absorption or emission spectrophotometry; Cu (2%), Pb (5%), and, at low concentrations, Mn (5%), Fe (5%) and Zn (5%), determined by graphite-furnace atomic absorption spectrophotometry; B (3%) determined by inductively-coupled-plasma emission spectrometry; Na estimated by charge balance. Analysis of Ge by R. A. Mortlock $\delta^{18}O$ relative to standard mean ocean water, contributed by W. C. Shanks III and J. K. Böhlke. H_2S endmembers in parentheses were estimated from weights of HgS in gas-tight samples. An H_2S endmember for Cl-depleted fluids collected in Ti-syringe samplers is not reported because, on several occasions, these gas-enriched samples were observed to de-gas (bubble) during ascent from the sea floor. Cl-enriched and Cl-normal data combined for estimation of B and $\delta^{18}O$ endmembers. ND, not determined.

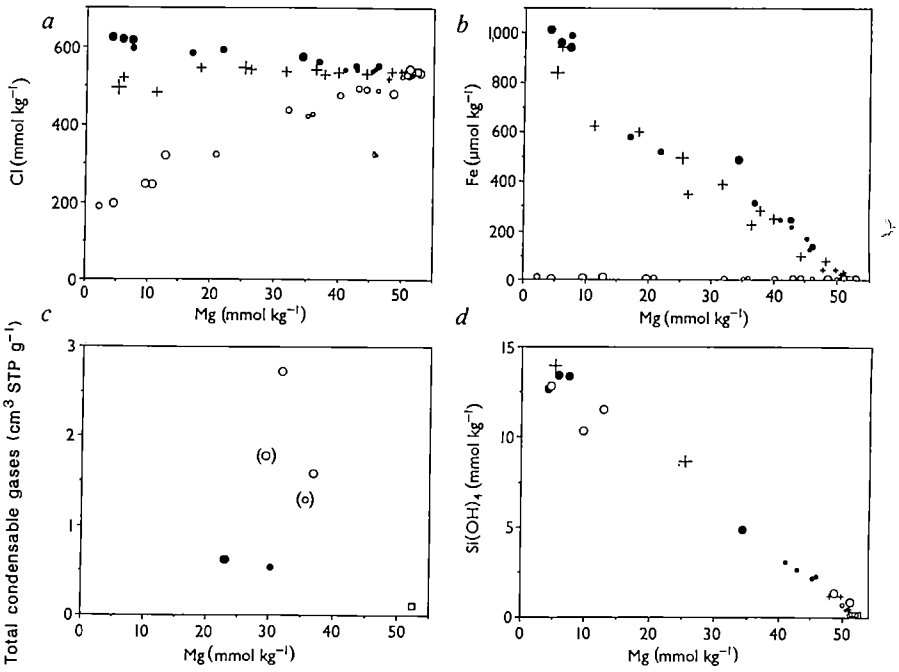

FIG. 2 Values of *a*, Cl (mmol kg^{-1}), *b*, Fe (μmol kg^{-1}) *c*, total condensable gases (cm^3 STP g^{-1}, assumed here to be CO_2; see text) and *d*, $Si(OH)_4$ (mmol kg^{-1}) plotted against Mg (mmol kg^{-1}) for ASHES vent fluids. Data symbols: ●, Inferno; +, Mushroom, Hell and Hillock; ○, Virgin Mound, Crack Vent, miscellaneous warm vents; □, sea water. Symbol sizes increase with each year group; 1986 smallest, 1988 largest. Total condensable-gas values in parentheses are from Crack Vent (higher value) or from a sample collected in a Ti-piston sampler (lower value) and were not considered in estimation of endmember. Missing data for $Si(OH)_4$ is the result of sample-storage artefacts. Because pure hydrothermal fluids are assumed to be totally depleted in Mg (ref. 14), hydrothermal endmember values are estimated by extrapolation of linear-regression lines for selected data trends to corresponding zero Mg concentrations.

in boiling conditions and the extent of phase segregation precludes strict comparison of our results to those in ref. 17, the trend and magnitude of gas partitioning at ASHES is very suggestive of phase separation.

Comparison of the Cl-depleted and Cl-enriched endmember values for Cl, Na, K, Br and Li yields a remarkably constant ratio of 0.293 ± 0.029. For Ca, however a 27% relative deficiency results. If we assume that the missing Ca has been removed by anhydrite precipitation during mixing of hydrothermal fluids with a small amount of cold sea water then the estimated composition of the Cl-depleted endmember is: 71% pure condensed vapour, 24% Cl-enriched fluid and 5% sea water. Application of a boiling fractionation model[17] to fluids initially at seawater chlorinity yields Inferno-level chloride concentrations

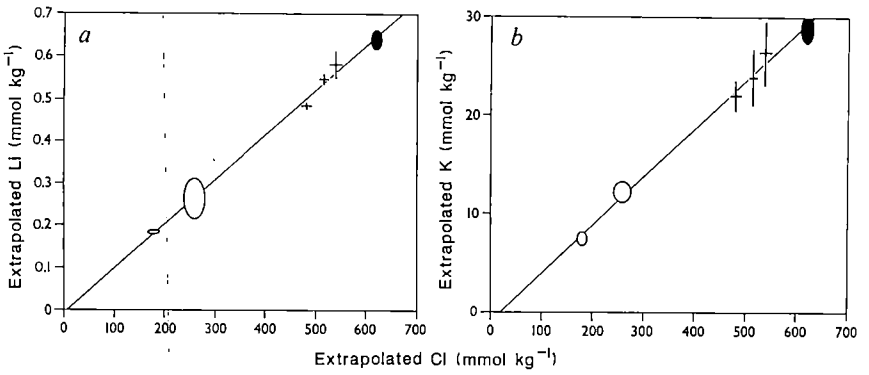

FIG. 3 *a*, Extrapolated Li (mmol kg^{-1}) and *b*, extrapolated K (mmol kg^{-1}) plotted against extrapolated Cl (mmol kg^{-1}) for high-temperature vents at ASHES. For *a*, $y=-0.006+0.00105x$; correlation coefficient $r=0.998$. For *b*, $y=-0.83+0.49x$; $r=0.997$. Vent symbols in order of increasing value: Virgin Mound, Crack Vent (both open symbols); Hillock, Mushroom, Hell (all crosses); Inferno (closed symbol). Discrete values obtained by extending lines formed between Li–Mg, K–Mg and Cl–Mg data pairs for sea water and sample to respective zero Mg concentrations. The average (centre point of symbols) and standard deviation (height and width of symbols $=2\sigma$) of pooled discrete values for each vent are shown. This presentation removes the seawater component from the sample mixtures and demonstrates for these species, which are conservative during seawater mixing, that all of the high-temperature fluids venting at ASHES can be described by a single mixing line that has, as one of its endmembers, fresh water like that expected to comprise the vapour phase during sub-critical phase separation[20].

after removal of ~20% of the water to the vapour phase, suggesting that Inferno fluids may be representative of a brine resulting from sub-critical phase separation (D.A.B. *et al.*, submitted). We note, however, that fluids with chlorinities both higher and lower than the extremes we have sampled may exist in the subsurface and that the magnitude of the Inferno Cl-enrichment could also be the result of fluid–rock interaction. Comparison of the venting distribution pattern to the self-consistent mixing behaviour of the ASHES fluids (Fig. 3) suggests that the venting endmembers are physically separated from one another well below the sea floor. The presence of Cl-depleted fluids within warm vents is consistent with studies of petroleum/gas reservoirs[23] and terrestrial hydrothermal systems[24], for which differential flow mechanics have been used to successfully model spatial segregation of flow phases (C. Fox, submitted).

The unexpectedly high $Si(OH)_4$, Ge and B values in the Cl-depleted fluids can be attributed to fluid–mineral reaction between the ascending vapour-enriched fluid and wall substrate and/or the high acid volatilities of these elements. Based on observations of B in geothermal systems[25], however, the expected contributions as a result of acid volatility would be insufficient to account for the observed levels in the Cl-depleted fluids, implying that fluid–mineral reaction is the major source of the enrichments. The $Si(OH)_4$ values for both endmembers are slightly supersaturated with respect to quartz with concentrations and temperatures suggesting an equilibration depth of 290 bar or 1.4 km below the sea floor assuming hydrostatic pressure[6]. Based on the CO_2 content of vesicles within Axial Volcano basalts, Dixon *et al.*[26] estimated that a magma chamber lay 2.7 km below the sea floor. Although these depth estimates may be poorly constrained, as is the depth of the proposed phase separation, a reaction path capable of producing the $Si(OH)_4$, Ge and B enrichments can be easily envisioned.

Phase-separation of hydrothermal fluids may have important consequences regarding subsurface ore deposition and delivery of metals to the deep ocean. The fact that regional hydrothermal plumes do not have as strong a metalliferous signature as plumes sampled elsewhere along the Juan de Fuca ridgecrest[27] may reflect the unusual source chemistry at ASHES. □

Received 19 April; accepted 14 July 1989.

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ACKNOWLEDGEMENTS. We thank the pilots and support personnel of *Pisces* and *ALVIN* for their assistance in the collection of samples. The Engineering Development Division of the Pacific Marine Environmental Laboratory, led by H. Milburn, designed and maintained the manifold sampling system. K. Roe, G. Lebon, D. Lambourn, M. Dudley, J. Day and G. Hall performed many of the measurements. K. Von Damm, C. Fox, and R. Feely provided valuable comment on the manuscript. This research was supported by the National Oceanic and Atmospheric Administration VENTS Program, the Ocean Sciences Division of the NSF, the Biology Section of The Office of Naval Research and the Geological Survey of Canada.

U–Pb baddeleyite ages for the Scourie dyke swarm, Scotland: evidence for two distinct intrusion events

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PRECISE and accurate radiometric ages for continental mafic dyke swarms are a prerequisite for global correlation of mafic magmatic events, calibration of apparent polar-wander paths and deciphering mechanisms of dyke emplacement. Precambrian dyke swarms, such as the Scourie dyke swarm in north-west Scotland, are invaluable time-markers, so that a precise and accurate knowledge of their emplacement age is critical when unravelling the complex geological evolution of many terrains. Precise dating is often difficult, however, because magma interaction with country rock and subsequent metamorphic events can severely perturb some isotopic systems. Recent advances in U-Pb geochronology[1–3] combined with the discovery that some mafic dykes contain trace amounts of uranium-bearing minerals such as baddeleyite (ZrO_2) and/or zircon have made it possible to obtain U–Pb ages for these rocks with a precision typically on the order of 1–2 Myr[4,5]. Some

of the first detailed U–Pb studies of Precambrian mafic dyke swarms[5,6] have shown that large volumes of mafic magma, 100,000 km^3, were emplaced into the continental crust in surprisingly short periods of time (<2 Myr). Here we report the results from a U–Pb study of three members of the Scourie dyke swarm, and find at least two periods of dyke emplacement at 2,418 and 1,992 Myr BP. We speculate on a potential global correlation of early Proterozoic mafic magmatism and hence on the origin of dyke swarms.

The Scourie dyke swarm consists of numerous, 10–100 m wide, mafic to ultramafic dykes that intrude the high-grade granulite- and amphibolite-facies gneisses of the Archaean Lewisian complex in north-west Scotland[7–9]. Four distinct dyke suites can be recognized based on petrological and geochemical criteria: Mg-rich bronzite picrites, norites, olivine gabbros and Fe-rich quartz dolerites, the latter being most abundant. From field evidence, the dolerites are most often older than the picrite, norite and olivine gabbro suites. But some dolerites do post-date the latter[7–9]. Unfortunately, field evidence allows only a very small proportion of the dykes to be placed in a relative chronological sequence. The dykes were emplaced between the late-Archaean Scourian tectonometamorphic events and the mid-Proterozoic Laxfordian events, which are characterized by strong shear deformation. In the central zone of the Lewisian complex, the granulite-facies metamorphism peaked at ~2.7 Gyr, biotite-pegmatites were emplaced at ~2.5 Gyr, followed by localized retrogression of the gneisses to amphibolite-facies assemblages (Inverian event)[10–12]. Most of the Scourie dykes were emplaced penecontemporaneously with this hydrous retrogressive phase, and many were affected by it, as well as by later Laxfordian metamorphism. The emplacement of the Scourie dyke swarm is therefore important in understanding the timing of the Scourian and Inverian events.

Because of its geological significance, the Scourie dyke swarm has been the subject of numerous geochronological investigations[13–15] and, until recently, a relatively precise Rb–Sr whole-rock age of 2,390±20 Myr (ref. 13), based on samples from three quartz dolerite samples, was considered to be the emplacement age for the swarm. Cohen *et al.*[15] challenged the validity of this Rb–Sr age, however, and proposed that the swarm is >300 Myr younger than this age, based on Sm–Nd internal isochrons for three relatively fresh dyke samples.

We collected large (≥30 kg) samples from many fresh dyke localities (some of these outcrops were sampled in the initial K–Ar study[14]) and most were searched intensively for baddeleyite and zircon. We took samples of all four petrogenetic types listed above and for most of these we sampled the coarser-grained central portions of the dykes. We found that three samples, representing members of the bronzite picrite, norite and olivine gabbro suites, contained sufficient quantities of baddeleyite/zircon for a U–Pb study. Two of these samples contained baddeleyite; a bronzite picrite (Beannach Dyke[12]) from a location 1 km west of Loch Assynt and an olivine gabbro from a dyke located 0.5 km south-west of Strathan (referred to here as the Strathan Dyke). At the Beannach Dyke sample locality, the dyke shows a primary petrological variation from orthopyroxene-rich margins to a plagioclase-rich centre[12] and, although different parts of the dyke were sampled to encompass the range of compositional variation, baddeleyite was only found in the central part, which is notably enriched in late-stage liquid. The olivine gabbro dykes typically show clinopyroxene-enriched margins, but baddeleyite was again found in the central portion of the Strathan Dyke. The zircon-bearing sample is a norite (N_1 of ref. 16) from Badcall Bay.

The U–Pb results for the baddeleyite and zircon fractions that were separated from these three samples are listed in Table 1 and are shown on concordia diagrams (Figs 1–3). As commonly

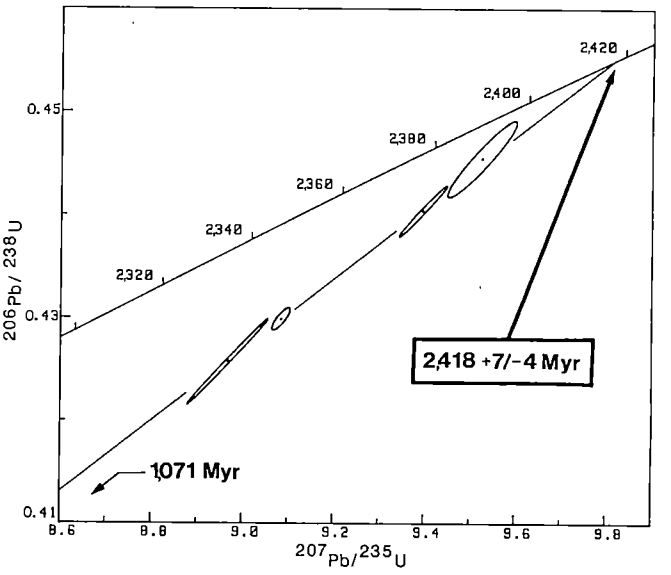

FIG. 1 U–Pb concordia diagram showing the results for four baddeleyite fractions from a member of the bronzite picrite suite (Beannach dyke).

TABLE 1 U–Pb results for accessory minerals separated from three Scourie dykes

Sample Number	Description †Fraction	weight (μg)	Concentration U (p.p.m.)	Pb (p.p.m.)	Sample/Total common Pb(pg)	Atomic ratios* $^{206}Pb/^{204}Pb$	$^{208}Pb/^{206}Pb$	$^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	$^{207}Pb/^{206}Pb$	Apparent age (Myr) $^{206}Pb/^{238}U$	$^{207}Pb/^{235}U$	$^{207}Pb/^{206}Pb$
Bronzite picrite (Beannach dyke)													
1	B, 5M, br	1	1,838	816	6	86,635	0.0029	0.4455 (9)	9.526 (18)	0.15509 (10)	2,375	2,390	2,403
2	B, 5M, br, (30)	6	2,476	1,089	25	20,307	0.0038	0.4404 (6)	9.396 (14)	0.15474 (3)	2,352	2,377	2,399
3	B, 5M, br, (17)	3	1,777	766	11	23,566	0.0228	0.4257 (21)	8.968 (44)	0.15278 (5)	2,287	2,335	2,377
4	B, 5M, br, (28)	3	1,652	715	6	>100,000	0.0175	0.4299 (5)	9.085 (9)	0.15326 (11)	2,305	2,347	2,383
Olivine gabbro (Strathan dyke)													
5	B, 5M, br, (16)	2	1,705	604	22	4,530	0.0045	0.3611 (4)	6.094 (7)	0.12241 (9)	1,987	1,989	1,992
6	B, 5M, br, (18)	2	1,744	617	38	2,402	0.0064	0.3559 (4)	6.004 (6)	0.12236 (7)	1,963	1,977	1,991
7	B, 5M, br, (31)	2	1,341	467	4	>100,000	0.0095	0.3581 (8)	6.041 (12)	0.12234 (14)	1,973	1,982	1,991
Norite, Badcall Bay													
8	Z, 3M, c, ab, (7)	2	376	96	29	436	0.1273	0.2103 (5)	3.919 (9)	0.13517 (12)	1,230	1,617	2,166
9	Z, 3M, c, ab	2	763	188	33	737	0.1043	0.2151 (3)	4.038 (6)	0.13616 (10)	1,256	1,642	2,179
10	R, 3M, gb, ab	48	12	4	48	267	—	0.2896 (21)	4.154 (38)	0.10402 (63)	1,640	1,665	1,697
11	R, 3M, or, ab	32	21	8	75	189	—	0.2888 (18)	4.125 (43)	0.10359 (81)	1,636	1,659	1,689

* Atomic ratios corrected for blank (Pb = 5 pg; U = 2 pg) and initial common Pb (ref. 19). All errors are quoted at 1σ and have been propagated to include most sources of uncertainty.
† Mineral analysed: B, baddeleyite; Z, zircon; R, rutile. Magnetic susceptibility: N, non-magnetic; M, magnetic at the indicated angle of side tilt on a Frantz isodynamic separator. For example, 3M refers to a magnetic fraction at three degrees side tilt. Colour: c, colourless; br, brown; gb, grey-brown; or, orange. ab, abrasion treatment[3]. A number in parentheses corresponds to the total number of grains analysed.

found in mafic dyke samples, the yield and grain size (longest dimension between 20–80 μm) of zircon and baddeleyite in the Scourie dyke samples were small and hence the weights of the fractions analysed (1–6 μg, Table 1) are small. The analytical procedures that we used for isolating U and Pb from baddeleyite and zircon and for measuring the U and Pb isotopic compositions on a VG354 mass spectrometer generally followed those in refs 2, 6 and 17.

The U–Pb results for four baddeleyite fractions from the Beannach Dyke are shown in Fig. 1. These four fractions define a discordia line (27% probability of fit) giving an upper-intercept age of 2,418+7/−4 Myr (2σ) and a lower-intercept age of 1,071 Myr. A striking feature of these baddeleyite analyses is the amount of discordance present (3.5–10.2%). The Beannach Dyke baddeleyite is unusual, however, in that many grains have a thin (5–20 μm thick) rim of polycrystalline zircon similar to that observed in some coronitic gabbros[18] and it is the presence of tiny amounts of zircon rim material that is largely responsible for the degree of baddeleyite discordance. Fractions 3 and 4 (Table 1), which show the greatest amount of discordance, contained baddeleyite grains with some visible zircon rim material. Furthermore, these same two fractions have noticeably higher $^{208}Pb/^{206}Pb$ ratios, reflecting a higher Th content in the zircon rim material. Unfortunately, the zircon rims are too thin to analyse separately so it is not possible to ascertain whether they are magmatic or a product of later metamorphism. A magmatic origin is perfectly feasible because the dyke is particularly fresh at this locality and it is also the most Zr-rich facies of the dyke. If the zircon rims are magmatic, the 2,418-Myr age represents the emplacement age of the dyke. If they are metamorphic, the emplacement age could be slightly older, to an extent depending on the age of zircon growth and the subsequent Pb-loss history.

The U–Pb results for the baddeleyite-bearing olivine gabbro dyke at Strathan are presented in Fig. 2. The three baddeleyite fractions (5–7, Table 1) have similar $^{207}Pb/^{206}Pb$ ages, between 1,992 and 1,991 Myr, and are between 0.3 and 1.8% discordant. No visible zircon rim material is present in this baddeleyite population. A best-fit discordia line through these analyses gives an upper-intercept age of 1,992+3/−2 Myr (78% probability of fit) and we interpret this as the emplacement age for this dyke.

We separated both zircon and rutile from the N_1 norite at Badcall Bay and the U–Pb results for two fractions of each mineral are shown in Fig. 3. The $^{207}Pb/^{206}Pb$ ages for the two

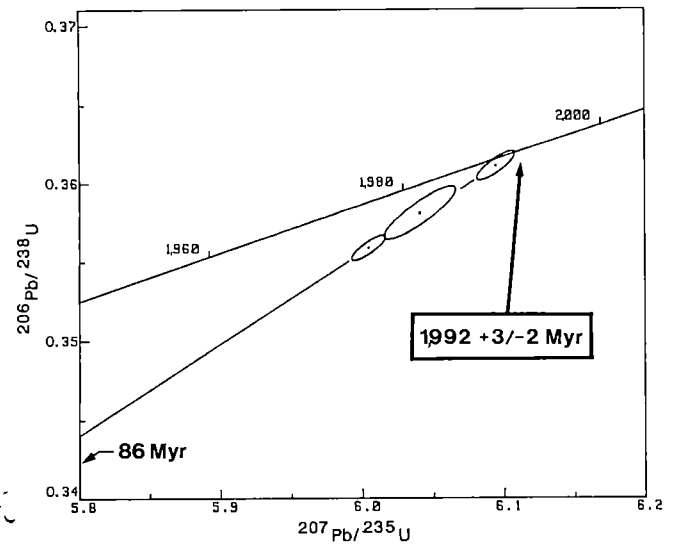

FIG. 2 U–Pb concordia diagram showing the results for three baddeleyite fractions from a member of the olivine gabbro suite (Strathan dyke).

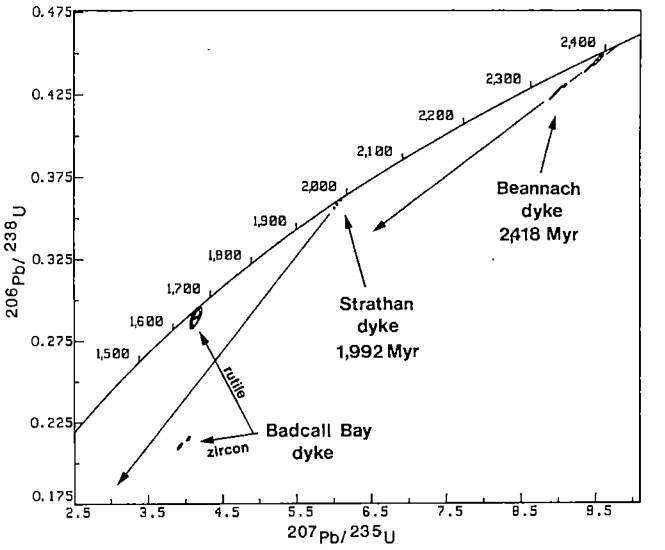

FIG. 3 Composite U–Pb concordia diagram showing the results for three Scourie dyke samples. Also shown are reference discordia lines for the Beannach and Strathan dykes with upper-intercept ages of 2,418 and 1,992 Myr, respectively.

zircon fractions are 2,166 and 2,179 Myr, respectively (fractions 8 and 9, Table 1) and provide a minimum estimate for the emplacement age of this dyke. Unfortunately, these two analyses are very discordant (>50%; see Fig. 3), even though they were given an air abrasion treatment[3], so an accurate U–Pb zircon age cannot be established. We note, however, that the zircon fractions have suffered a significant amount of Pb-loss during a relatively recent event. Assuming the emplacement age for the norite dyke is identical to the Beannach Dyke (~2,420 Myr), with which it has petrogenetic affinity, then the projected lower-intercept age is ~440 Myr. A possible cause for the substantial amount of Pb-loss in the two zircon fractions is tectonic disturbance related to early movements along the Caledonian Moine thrust zone. Many olivine-bearing dykes close to this thrust zone have in fact suffered late-stage serpentinization, indicating fluid activity at this time.

The two rutile fractions from the fresh Badcall Bay norite are also shown in Fig. 3 and have $^{207}Pb/^{206}Pb$ ages of 1,697 and 1,689 Myr. Considering the nearly concordant nature of these analyses, it is evident that rutile was either formed, or the U–Pb systematics reset, after emplacement of even the youngest known Scourie dyke at 1,992 Myr. In either case, the ~1,700-Myr rutile age is clearly the result of a later Laxfordian metamorphic overprint. We note that the norite dyke is situated much closer to the Laxford metamorphic front than the olivine gabbros and picrites, which are located 15–20 km south of the front. Moreover, an extension of this norite (N_2) only 30 m away, is completely altered and is cross-cut by a Laxfordian muscovite pegmatite.

These U–Pb baddeleyite results provide the first convincing evidence for at least two distinct episodes of Scourie dyke emplacement. They demonstrate that caution should be exercised in assuming that all dykes in the same location with similar orientations have identical emplacement ages. The results from previous petrogenetic studies[9,12] indicate that the norites and picrites have been derived from the same, rather refractory, mantle source whereas the olivine gabbros and quartz dolerites have been derived from a separate, more Fe-rich, mantle source. The U–Pb data are consistent with this idea because the bronzite picrite suite and probably the norite suite are part of an older dyke emplacement (~2,418 Myr) whereas the olivine gabbro suite was emplaced more than 400 Myr later, at 1,992 Myr.

The U–Pb minimum age of 2,418 Myr for the Beannach Dyke

is slightly older than the previously reported Rb–Sr whole-rock age of 2,390 ± 20 Myr based on three dolerites from the Scourie area[13], commonly accepted hitherto as the age of the entire swarm. If the Rb–Sr age was determined from pre-picrite dolerites then it may be regarded as anomalously young. The U–Pb baddeleyite age of 1,992 Myr for the Strathan Dyke generally agrees with the Sm–Nd mineral ages of 2,031–1,983 Myr determined for three separate fresh dolerite samples from the Scourie area[15]. Although the U–Pb results are generally consistent with both the previous Rb–Sr and Sm–Nd studies, the U–Pb baddeleyite results clearly demonstrate the presence of two temporally distinct periods of dyke intrusion in the Lewisian and resolves the controversy surrounding the previous age determinations. Unfortunately, the lack of baddeleyite in the fresh dolerites that we have sampled prevents any assessment by U–Pb techniques of the proportion of dolerites that might belong to the ~2400- or ~2000-Myr suites. In addition, the U–Pb results for accessory minerals from the norite dyke at Badcall Bay are evidence for disturbances in the U–Pb system related to both the later Laxfordian metamorphic episode (new growth or resetting of rutile) and Caledonian events (Pb-loss in zircon).

We stressed earlier that precise and accurate U–Pb baddeleyite ages for mafic dyke swarms are a prerequisite for global correlation of mafic magmatic events. Although precise ages are not yet available for most swarms, it is clear that mafic dyke swarms are a common feature of most Precambrian cratons worldwide with individual cratons containing numerous discrete dyke suites. One of the most promising techniques for correlating dyke swarms on a global scale is by using U–Pb baddeleyite geochronology. Interestingly, the 2,418-Myr age for the Scourie picrite suite is close to a recently determined U–Pb baddeleyite/zircon age of 2,452 + 3/−2 Myr for the Hearst–Matachewan swarm in North America[5]. Although correlation over such distances is necessarily speculative, we note that the least discordant results, from the Beannach Dyke baddeleyite that contained no visible zircon rim material, are also consistent with an emplacement age of ~2,450 Myr, with subsequent development of zircon rims during Laxfordian metamorphism (~1,800 Myr) and a small component of relatively recent Pb-loss in the zircon. If this correlation can be further substantiated, then the origin of such widespread mafic magmatism requires consideration of processes within the Earth that operate globally, rather than origins for all dyke swarms being totally dependent on local tectonic constraints. Future application of precise U–Pb geochronology combined with other detailed studies on a variety of aspects of mafic dykes including efforts to obtain 'primary' initial Sr–Nd–Hf–Pb isotope information on accurately-dated dykes may help to elucidate the origin, significance and extent of temporally related major Precambrian mafic dyke swarms. □

Received 29 June; accepted 27 July 1989.

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ACKNOWLEDGEMENTS. We would like to express our gratitude to M. Moore for help with sample preparation and F. Corfu for his comments on an early version of the manuscript. Comments by P. Taylor are also appreciated.

High-resolution leaf-fossil record spanning the Cretaceous/Tertiary boundary

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THEORIES that explain the extinctions characterizing the Cretaceous/Tertiary (K/T) boundary[1–3] need to be tested by analyses of thoroughly sampled biotas. Palynological studies are the primary means for stratigraphic placement of the terrestrial boundary and for estimates of plant extinction[4–12], but have not been combined with quantitative analyses of fossil leaves (megaflora). Megafloral studies complement palynology by representing local floras with assemblages capable of high taxonomic resolution[13], but have previously lacked the sample size and stratigraphic spacing needed to resolve latest Cretaceous floral history[5,14–18]. We have now combined megafloral data from a 100-m-thick composite K/T boundary section in North Dakota with detailed palynological analysis. Here the boundary is marked by a 30% palynofloral extinction coincident with iridium and shocked-mineral anomalies and lies ~2 m above the highest dinosaur remains. The megaflora undergoes a 79% turnover across the boundary, and smaller changes 17- and 25-m below it. This pattern is consistent with latest Cretaceous climatic warming preceding a bolide impact.

The badlands of the Little Missouri River near Marmarth, southwestern North Dakota, expose extensive fossiliferous outcrops of the Upper Cretaceous (Maastrichtian) Hell Creek Formation and the lower Palaeocene Ludlow Member of the Fort Union Formation[19–21]. The Hell Creek is a 110-m-thick unit of sandstone and mudstone representing channel- and floodplain-soil deposits of a meandering fluvial system[19]. It is conformably overlain by the 100-m-thick Ludlow Member, which consists of lignite, lenticular and tabular sandstone bodies and laminated mudstone. The Ludlow represents fluvio-deltaic deposition on the western margin of the Cannonball seaway of Danian age[20,21]. The Hell Creek–Ludlow contact is placed at the base of the lowest lignite. Dinosaur remains are common in the Hell Creek; their highest recorded appearance is 1 m below the base of the Ludlow Member (Milwaukee Public Museum, locality 1056).

Megafloral remains ($n = 11,503$), were collected at 87 localities from the base of the Hell Creek to near the top of the Ludlow, in a 25 × 65-km field area centred in Marmarth. For each locality, stratigraphic position was measured, sedimentary facies analysed and a census made to assess the relative abundance of megafloral taxa. Because of the chaotic state of Cretaceous angiosperm taxonomy, a morphotype system was established to assure reproducible identifications[22]. Megafloral data were plotted for each of the localities within 50 m of the formational contact that yielded >20 specimens ($n = 8,428$; Fig. 1).

At Pyramid Butte, 12 km north of Marmarth, a 1.9-m section across the basal Fort Union lignite and adjacent strata was sampled in contiguous 10-cm intervals for neutron activation and palynological analyses (Fig. 2).

Neutron activation analyses revealed an iridium anomaly of 0.34 parts per 10^9 (p.p.b.) over background of 0.025 p.p.b. in a 10-cm sample including dark-grey mudstone and sandstone at the top of the 90-cm-thick lignite (Fig. 2). Subsequent sampling at 2–3-cm intervals showed an anomaly of 0.72 p.p.b. restricted to the mudstone. The mudstone also contains rare shock-metamorphosed mineral grains similar to those found at other K/T boundary sites[9,23].

Palynological analyses show that ~30% of the Upper Cretaceous palynoflora, including characteristic Maastrichtian taxa, is absent above the level of the iridium and shocked-mineral anomalies (Fig. 2). Palynomorph diversity decreases from ~90 taxa in the top metre of the Cretaceous strata to ~60 taxa in the basal 80 cm of the Palaeocene strata. The fern-spore abundance 'spike'[10,24,25] that characterizes several other K/T boundary sections was not found at Pyramid Butte, indicating either that it was not deposited, as seems to be the case in several sites in Canada[26], or that it was subsequently removed. Minor disturbance of the boundary interval is suggested by the unconformable base of the overlying, 10-cm-thick, laterally discontinuous sandstone bed and common sand-filled burrows of, possibly, insects in the iridium-bearing mudstone and the top of the lignite. This may account for the small iridium anomaly, the relative rarity of shocked grains and the absence of the characteristic 'boundary clay layer'[8,23].

Significantly, the palynologically defined K/T boundary and associated anomalies at Pyramid Butte occur above the basal lignite, some 90 cm into the Fort Union Formation. At all other published K/T sites in the northern Great Plains[9,10,12], the boundary occurs at, or just below, the base of a lignite bed. This relationship was interpreted as evidence of an impact-related increase in precipitation and the resulting deposition of peat[18]. The Pyramid Butte site shows that the Hell Creek–Ludlow facies transition began before the K/T transition, and implies different causes for the regional start of peat deposition and the K/T extinctions.

The megafloral data from Marmarth represent the first quantitative distribution of megaflora into zones in uppermost Cretaceous strata. Three zones are described here, two from the upper 50 m of the Hell Creek Formation and one from the basal 50 m of the Fort Union Formation (Fig. 1). The lowest, HC II, is divided into two subzones. The lower subzone, HC IIa, is dominated by *Dryophyllum subfalcatum* (43%) and *Platanophyllum montanum* (13%). Total diversity is 80 taxa (2,047 specimens, 12 localities). The overlying subzone, HC IIb, is dominated by '*Vitis*' *stantonii* (66%) and *D. subfalcatum* (14%). Total diversity is 12 taxa (1,044 specimens, 6 localities).

Only 25% of the HC II taxa persist into the uppermost Hell Creek zone, HC III, but much of this change is due to gradual turnover within HC II. Dominant taxa in HC III are *Dombeyopsis trivialis* (29%) and '*Cissus*' *marginata* (15%) (Fig. 1). Total diversity is 63 taxa (1,741 specimens, 17 localities). The HC III megaflora is unusual in that it contains many taxa previously known from the Upper Cretaceous of the Raton basin, 1,000 km to the south[17,27]. Their presence in North Dakota probably represents northward migration of a thermophilous flora (including palms and Laurales) in response to a warming climate. Latest Cretaceous climatic warming is further supported

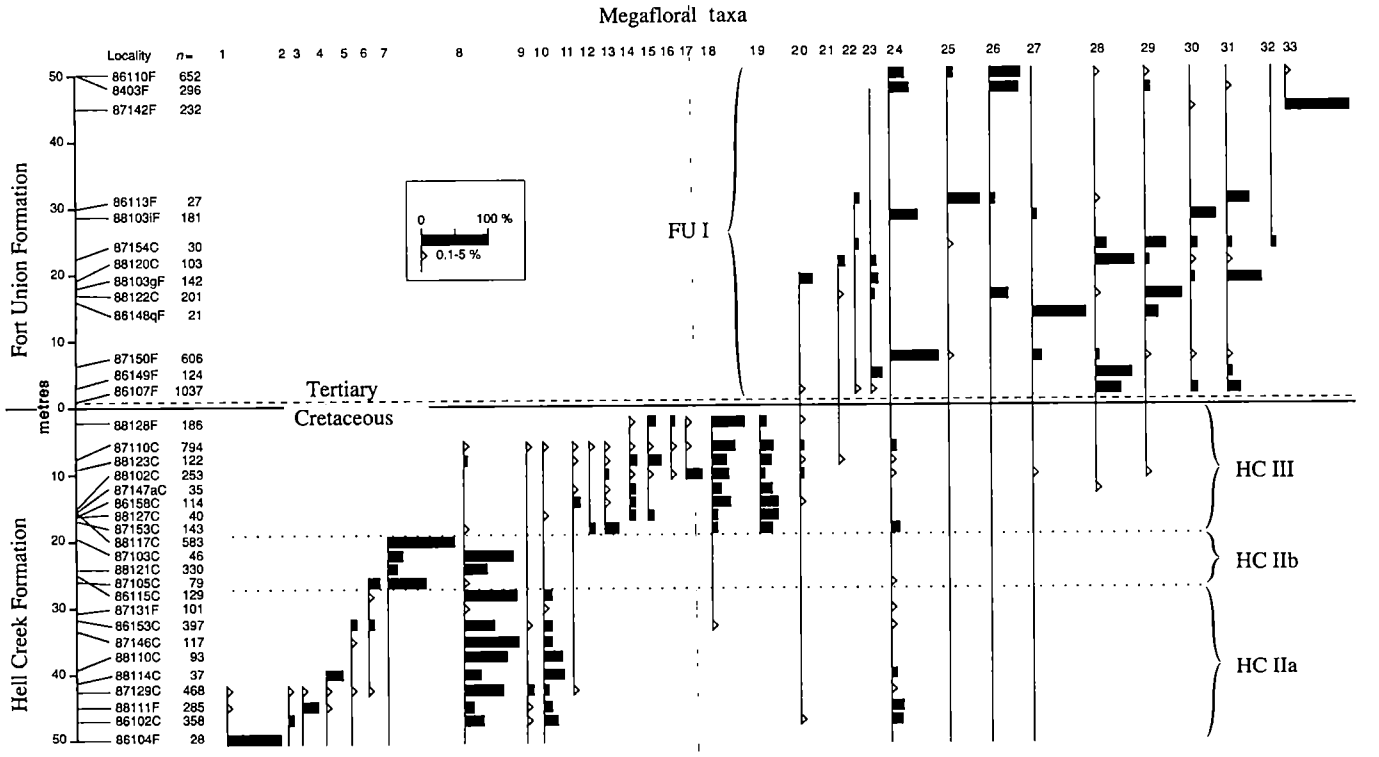

FIG. 1 Stratigraphic ranges and relative percentage histograms of megafloral taxa relative to the Hell Creek–Fort Union contact (solid line), the palynologically defined K/T boundary (dashed line) and megafloral zone boundaries (dotted lines). All taxa that account for 5% or more of the flora at any level and occur at two or more levels are plotted. The facies of each locality is noted by letter: C, channel, F, floodplain. Range lines that terminate with no tick marks represent taxa from Marmarth that occur outside the stratigraphic range of this chart or that occur at localities not plotted. Localities 86115 and 88117 are plotted out of stratigraphic sequence because they occur in a section that appears to have been compressed by a local hiatus. The taxa (and morphotype reference numbers) are: 1-*Pistia corrugata* (HC77), 2-*Cercidiphyllum ellipticum* (HC73), 3-*Ginkgo adiantoides* (HC114), 4-"*Ficus*" *artocarpoides* (HC86), 5(-aff.)Magnoliaceae (HC66), 6(-aff.)Dilleniaceae (HC62), 7-"*Vitis*" *stantonii* (HC14, HC108), 8-"*Dryophyllum*" *subfalcatum* (HC49), 9-*Trochodendroides nebrascensis* (HC103), 10-*Platanophyllum montanum* (HC57), 11-(aff.)Platanaceae (HC2), 12-*Paleoaster iniquirienda* (HC7), 13-(cf.)"*Dryophyllum*" *tennessensis* (HC44), 14-*Paranymphaea hastata* (HC111), 15-Laurales (HC162), 16-Laurales (HC163), 17-"*Liriodendron*" *laramiense* (HC166), 18-*Dombeyopsis trivialis* (HC105), 19-"*Cissus*" *marginata* (HC1, HC106), 20-Palmaceae (FU37), 21-(aff.)Hamamelididae (FU76), 22-Flacourtiaceae (FU49), 23-(aff.)Cornaceae (FU31), 24-Taxodiaceae including *Glyptostrobus europaeus* (FU4, HC9), *Metasequoia occidentalis* (FU3, HC35), *Taxodium olrikii* (HC71), *Sequoia sp.* (HC70), and unidentified taxodiaceous foliage, 25-*Cercidiphyllum genetrix* (FU5), 26-"*Cocculus*" *flabella* (FU43), 27-*Cupressinocladus interruptus* (FU26) 28-"*Populus*" *nebrascensis* (FU7), 29-*Platanus raynoldsii* (FU16), 30-*Paranymphaea crassifolia* (FU1), 31-*Dicotylophyllum anomalum* (FU29), 32-unidentified dicotyledon (FU47), 33-*Quereuxia angulata* (FU2). Aff., affinity (implies similarity); cf., compare (implies a closer similarity).

by physiognomic analysis of these megafloras that shows an increased percentage of dicotyledonous angiosperms with entire-margined leaves (from 35% to 49%) on transition from HC IIa to HC III (ref. 28).

Megafloral turnover at the K/T boundary in southwestern North Dakota is extremely high and seems to represent major extinction. The HC III megaflora is replaced by basal Palaeocene megaflora FU I. Because pollen and leaves are derived from the same vegetation, megafloral change is probably as abrupt as that represented by the palynoflora. Because similar facies (channel, floodplain) above and below the K/T boundary produce different mega- and palynofloras, and Cretaceous palynomorphs occur throughout the basal Fort Union lignite, it is unlikely that floral change is an artefact of facies or collecting bias (Fig. 2). Only 21% of the HC III taxa, including no dominants, survived into the Palaeocene. Zone FU I extends from Alberta to Colorado[15,29-31]. Near Marmarth it begins at the K/T boundary and is dominated by taxodiaceous conifers (20%), '*Populus*' *nebrascensis* (16%), '*Cocculus*' *flabella* (12%), *Dictotylophyllum anomalum* (9%), and *Paranymphaea crassifolia* (5%). The total diversity is 72 taxa (3,795 specimens, 30 localities). The lowest FU I locality (86107) occurs 15 cm above the iridium anomaly at Pyramid Butte and has a diversity of 29 megafloral taxa (1,037 specimens). Consequently, prolonged existence of a low-diversity, post-impact, 'recovery' flora[17,18] seems unlikely.

For both palynoflora and megaflora, the K/T boundary marks the most distinct change in the section. Although the palynological analysis concentrated on the 1.9 m boundary interval, other data from the Hell Creek indicate that the entire formation lies within the *Wodehouseia spinata* Assemblage Zone[32]. Subdivisions of this zone paralleling megafloral zones HC IIa–HC III are not clearly evident. These differences probably result from different taxonomic resolution and taphonomic pathways of the plant organs[13] and support an integrated approach based on the micro-stratigraphic resolution of palynomorphs and the high taxonomic resolution of megaflora. The disappearance of the uppermost Cretaceous megaflora, and its replacement by the widespread basal Palaeocene megaflora, in context with abrupt palynological extinctions, shocked minerals, and the iridium anomaly, supports the theory that floral extinctions at the K/T boundary were caused by the impact of an extraterrestrial body. □

Received 7 April; accepted 14 July 1989.

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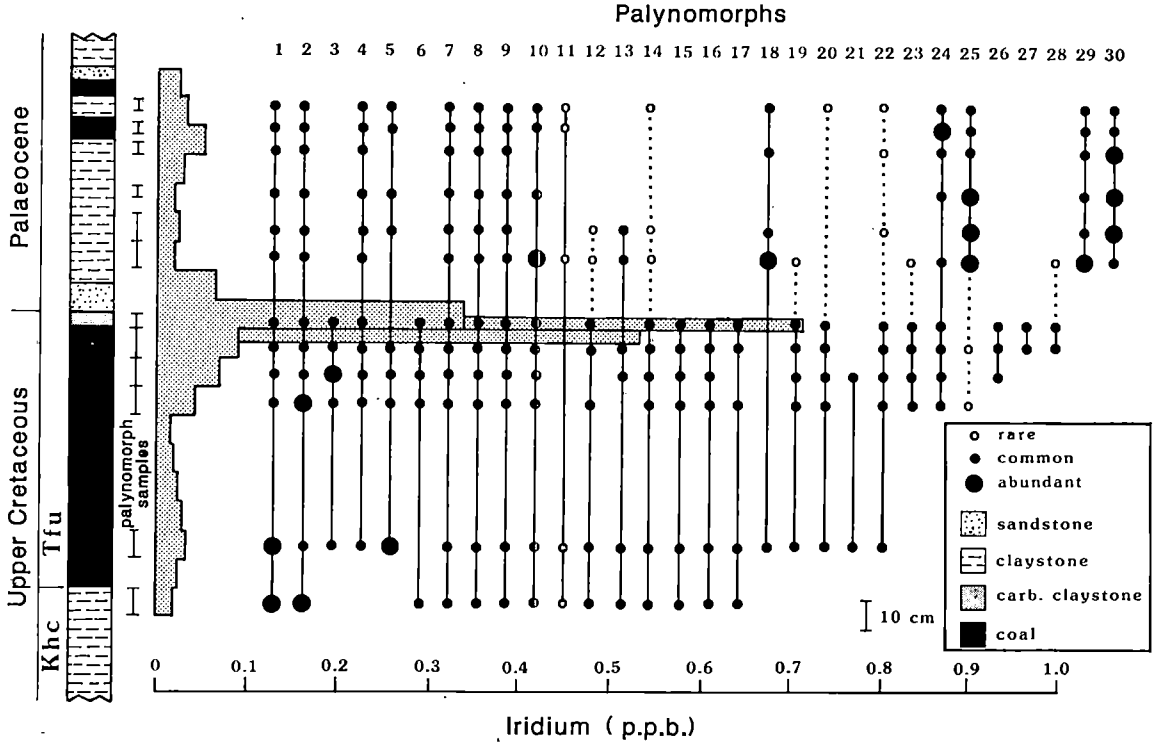

FIG. 2 Distribution and relative abundances of iridium and the most common palynomorphs in the 1.9-m-thick K/T boundary section at Pyramid Butte, North Dakota. Khc, Hell Creek Formation; Tfu, Fort Union Formation (Ludlow Member); histogram shows iridium concentrations in p.p.b. measured in continuous 10-cm-interval samples and the maximum anomaly from additional samples at smaller intervals; peak iridium anomaly is coincident with shocked mineral occurrence and disappearance of characteristic Upper Cretaceous palynomorphs; vertical lines show ranges of palynomorphs within sampled intervals; dotted lines extend ranges on basis of very rare, non-typical (possibly reworked) occurrences. Palynomorphs are grouped by taxonomic category: bryophytes and pteridophytes, 1–3; gymnosperms, 4–6; angiosperms, 7–30. The taxa are: 1-*Laevigatosporites* spp., 2-*Stereisporites* spp., 3-*Osmundacidites* sp., 4-*Taxodiaceaepollenites hiatus*, 5-(cf.)*Glyptostrobus* sp., 6-*Ephedra multipartita*, 7-*Arecipites tenuiexinous*, 8-*Triporopollenites* spp., 9-*Ulmoideipites* spp., 10-*Kurtzipites trispissatus*, 11-*Momipites inaequalis*, 12-*Tricolpites parvistriatus*, 13-*Wodehouseia spinata*, 14-*Proteacidites* spp., 15-*Libopollis jarzenii*, 16-*Liliacidites complexus*, 17-*Myrtipites granulatus*, 18-*K. circularis*, 19-*Aquilapollenites* n. sp. 20-*A. quadrilobus*, 21-*Cranwellia rumseyensis*, 22-*T. microreticulatis*, 23-*Orbiculapollis lucidus*, 24-*T. hians/parvus*, 25-*Ericaceoipollenites rallus*, 26-*A. delicatus* var. *collaris*, 27-*Leptopecopites pocockii*, 28-*A. reticulatus*, 29-*Pandaniidites typicus*, 30-*Syncolporites minimus*. n. sp., new species; sp., species; spp., more than one such species; var., variety.

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ACKNOWLEDGEMENTS. We thank L. J. Hickey, D. E. Fastovsky, R. F. Fleming, L. Raubeson, D. W. Taylor, R. Thompson, G. R. Upchurch Jr, E. S. Vrba, S. L. Wing and J. A. Wolfe for helpful comments; Milwaukee Public Museum for dinosaur locality data, and G. Izett for analysis of shocked mineral grains. Funding was supplied by Yale University, Geological Society of America, and Sigma Xi.

Orb-web weaving spiders in the early Cretaceous

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THE use of a snare woven from spun silk as a means of capturing prey is the most outstanding achievement of spiders. Fossil spider spinnerets are known from the Devonian[1] and Carboniferous[2] periods. Presented here, however, is evidence of the antiquity of the use of woven silk in prey capture: spider fossils showing morphological adaptations for web weaving, from the Lower Cretaceous lithographic limestone of the Sierra de Montsech, north-east Spain. Reflected light microscopy reveals details of the pattern and structure of the tarsal claws, which were adapted for the handling of silk and locomotion on a web. As only two Mesozoic spiders, *Juraraneus* and *Jurarchaea* from the Jurassic of the Soviet Union[3], have been formally described, the four specimens reported here are an important addition to the fossil record. These belong to three new genera placed in the modern superfamilies Dinopoidea and Araneoidea. Members of both superfamilies weave orb webs or orb-web derivatives[4]. The Montsech spiders preyed on the abundant insect life which is also preserved in the Cretaceous lithographic limestone[5].

The Lower Cretaceous lithographic limestone of the Sierra de Montsech, Lérida Province, north-east Spain, is renowned for its excellent preservation of land plants, crustaceans, insects, fish, amphibians, reptiles and birds[5,6]. Four specimens of spiders are among the collections made in recent years at the quarries of La Cabrua (specimens LC 1150 IEI, LC 1753 AP, LC 1754 AP) and La Pedrera de Meia (LP 1755 AP). Locality details are given in ref. 7 and specimens are deposited in the Instituto de Estudios Ilerdenses, Lérida. The fifty-metre succession of limestone exposed in the quarries has been determined as late Berriasian to early Valanginian in age on the basis of microfossils[6,8] The fine-grained, thinly bedded limestones bearing the spider fossils represent a lagoonal or lacustrine environment between the Ebro continent to the south and marine conditions to the north[6]. The spiders are preserved as brittle, brown cuticle, and morphological details are seen best where the cuticle is covered by a thin layer of translucent matrix. Reflected light microscopy, using oil-immersion objectives, enables observation at high magnification. All three new genera belong to the suborder Araneomorphae; formal taxonomy of the new taxa will be published elsewhere[9] and only brief descriptions follow here.

LC 1150 IEI (Fig. 1) is referred to the Araneoidea. This superfamily is inadequately defined at present[10], but features include: lack of synapomorphies of other groups, serrate setae, paracymbium, few trichobothria, web-weaving and globose abdomen. Although none of these characters are unique to Araneoidea, their combined presence in LC 1150 IEI suggest its inclusion in this superfamily. The subelliptical carapace bears a raised cephalic area and no fovea. The abdomen (globular in life) bears a compact group of short spinnerets at the posterior. The sternu... is subtriangular and there is a small, subtriangular labium. Serrate setae are present and no trichobothria can be seen on the specimen. The chelicerae are large (0.4 times the carapace length), forwardly directed and bear inner and outer rows of denticles and a mesal ridge. The specimen is an adult male, bearing a modified palp with a long embolus and what appears to be a small, proximal paracymbium. The legs are

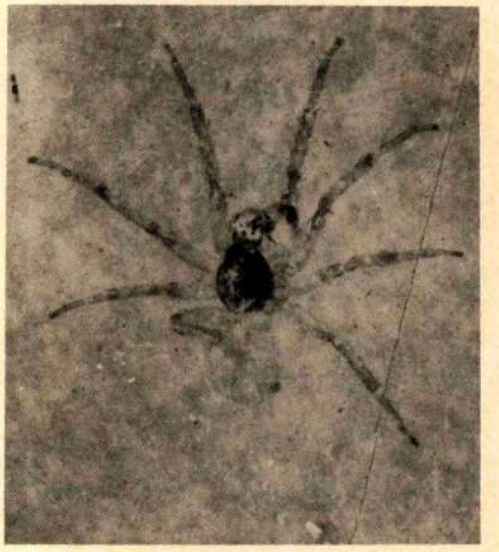

FIG. 1 Araneoid spider from the Lower Cretaceous of Montsech, Spain (LC 1150 IEI). Specimen compressed to the left; carapace offset to left, revealing right coxae; left male palp curved round anterior of left chelicera and showing long embolus, right palpal bulb lying on right leg 1; spinnerets appear as dark patch in lower right of abdomen (compressed to the left). Magnification, ×5.2.

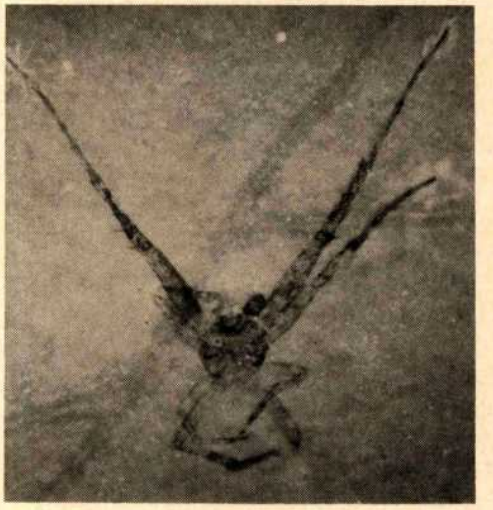

FIG. 2 Araneoid spider from the Lower Cretaceous of Montsech, Spain (LC 1753 AP). Note subcircular carapace, right male palp with planospirally coiled embolus, elongate legs 1 and 2, abdomen absent in this specimen. Magnification, ×3.2.

subequal in length (formula, shortest to longest, 1243) and are about 3 times the length of the carapace. The tarsi bear pectinate paired claws, a small, non-pectinate median claw and numerous serrate bristles (Fig. 4*a*). It is impossible to assign the specimen to a family on these characters, but a small, sheet-web weaving araneoid (such as linyphiid or theridiid) is suggested.

LC 1753 AP (Fig. 2) and LC 1754 AP belong to the same species, which is referred to the Araneoidea. The foveate carapace is slightly wider than long and the oval abdomen bears a subterminal group of short spinnerets. Serrate setae occur and trichobothria are present on the superior prolateral surface of femora 3 (double row) and 4 (single row). The sternum is circular. Both specimens are adult males bearing modified palps with a planospirally coiled embolus. The leg formula is 1243, leg 1 being 6 times the length of the carapace and more than twice the length of leg 3. The paired tarsal claws bear six teeth, the median claw is long, curved and not pectinate and accessory claws are present (Fig. 4*b*). The pattern of elongated anterior legs, short third legs and femoral trichobothria occurs as a convergent phenomenon in two extant spider families: the Uloboridae (Dinopoidea) and the Araneidae (Araneoidea). Both groups are wrap-attack orb-web weavers, the former using cribellum and calamistrum to produce non-viscid 'cribellate' silk in contrast to the viscid silk of araneids. LC 1753 AP and LC 1754 AP lack characters that would refer them to the Dinopoidea (see below) and appear to be closest to the Argiopinae within the family Araneidae.

The carapace of LP 1755 AP (Fig. 3) is oval, has no well-defined fovea and just posterior to the midline is a break of slope which accommodated the forwardly extended abdomen in life. A compact group of six spinnerets and a cribellum is present subterminally on the abdomen. The leg formula is 1243; leg 1 is more than 5 times the length of the carapace and more than twice the length of leg 3. Plumose setae are present and there are many trichobothria on what appears to be the retrolateral surface of femur 2 and possibly the prolateral surfaces of femora 3 and 4. The paired tarsal claws are small and lack teeth, the median claw is long and probably lacks teeth (if present, they are minute). A pair of large accessory claws is present on the tarsi. The superior surface of metatarsus 4 is curved and bears a calamistrum. The palps are unmodified, so this is a female or immature specimen. LP 1755 AP belongs in the superfamily Dinopoidea, possessing calamistrum and cribellum, plumose setae and a characteristic tarsal claw pattern; it lacks the tarsal macrosetae and feathery setae of uloborids[11], and is therefore not placed in that family.

All three Montsech spider genera possess three tarsal claws with the characteristic serrate accessory claws adapted for web-weaving[12]. The combination of elongated anterior legs, short third legs and femoral trichobothria in LC 1753 AP, LC 1754 AP and LP 1755 AP occurs in only two groups of living spiders, Uloboridae and Araneidae, both of which are weavers of orb webs. These spiders rest in characteristic positions[13], generally with the anterior legs outstretched, and the short third legs gripping a twig. The function of the femoral trichobothria is not understood, but the organs occur, as in the fossil spiders, on the retrolateral surface of forwardly directed femora and prolateral surfaces of backwardly directed femora. Thus, at rest, the trichobothria point to the lateral sides of the animal.

There is controversy over whether the orb web is a convergent phenomenon in Araneoidea and Dinopoidea, or whether it

FIG. 3 Dinopoid spider from the Lower Cretaceous of Montsech, Spain (LP 1755 AP). Specimen is dorsoventrally compressed, dorsal and ventral surfaces superimposed. Note elongate legs 1 and 2, dark line on superior (posterior in specimen) side of metatarsus 4 indicates calamistrum; abdomen (wrinkled posteriorly) with ventral group of small spinnerets, transverse line immediately anterior marks cribellum. Magnification, ×7.4.

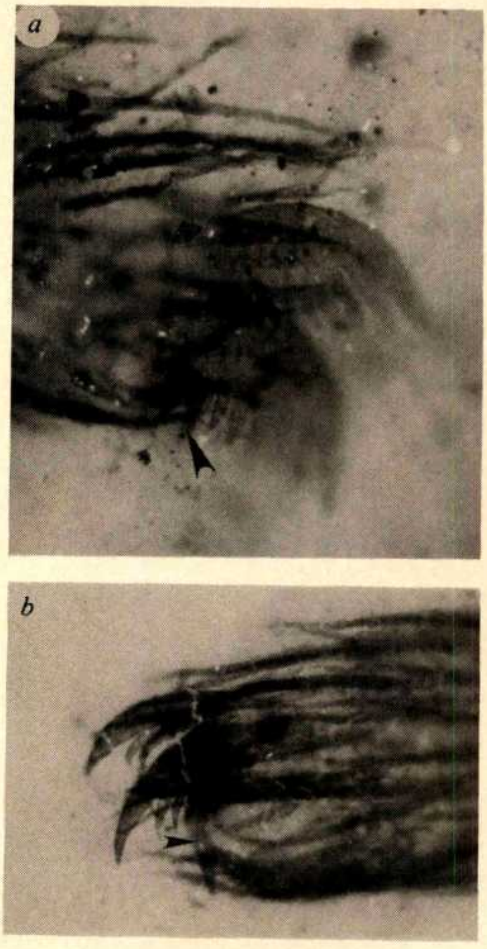

FIG. 4 *a*, Tarsal claws on left leg 2 of araneoid spider LC 1150 IEI. Note large, pectinate paired claws, small, uncinate median claw (arrowed), serrate accessory claws (bottom) and bristles. Magnification, ×290. *b*, Tarsal claws on right leg 2 of araneid spider LC 1753 AP. Note pectinate paired claws, median claw (arrowed), and large, serrate accessory claws (bottom) and bristles. Magnification, ×250. In web-weaving spiders, the silken thread is pulled by the median claw on to the serrations of the accessory claws; the median claws take no part in normal handling of silk[15]. Spiders which do not weave prey-capture webs do not possess accessory claws, and the median claw may be absent or greatly reduced.

evolved only once[10]. The Montsech spiders provide evidence that the two groups of orb-web weavers were already well defined by the early Cretaceous. No tarsal claw details are preserved in the Jurassic araneoid *Jurararaneus*[14]; nevertheless, it may have been an orb-web weaver. If the orb web evolved only once, in the common ancestor of Araneoidea and Dinopoidea[4], its origin lies in the Jurassic or earlier. □

Received 10 April; accepted 21 July 1989.

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ACKNOWLEDGEMENTS. I thank Antonio Lacasa-Ruiz of the Instituto de Estudios Ilerdenses, Lérida, for bringing these specimens to my attention and loaning them for study, and John Dalingwater for help with photography and for commenting on the manuscript.

Interspecific competition increases local extinction rate in a metapopulation system

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THE importance of interspecific competition for the distribution and abundance of organisms has been hotly debated during the last decade[1–7]. Although many field experiments have shown effects of interspecific competition on abundance and reproduction[1,3], there is no unequivocal experimental evidence that interspecific competition can influence rates of local extinction in the field. Here I report that in a long-term field experiment with artificial rockpools, interspecific competition between three common rockpool zooplankton species led to increased local extinction rates. In addition, studies of the distributional dynamics of the species in natural rockpools also showed that interspecific competition increases extinction rates. These results imply that interspecific competition is likely to limit the regional richness of species in the rockpool metapopulation system. MacArthur and Wilson[8] were the first to suggest that an increase in extinction rate per species with an increase in the number of species could influence species richness on islands. Moulton and Pimm[9] found that this was so among birds introduced to the Hawaiian islands, but the present study is the first field experiment providing unequivocal evidence of the effect.

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Rockpools, small bedrock depressions containing fresh or brackish water, are common in coastal areas around Scandinavia. The most abundant small crustaceans in rockpools are three waterflea species, *Daphnia magna*, *D. pulex* and *D. longispina* (Cladocera)[10–15], which coexist regionally along the coasts of Sweden[11] and Finland[12–15]. The smallest rockpools containing *Daphnia* have a volume of ~20 litres, and the largest ~10^5 litres. The niches of the species widely overlap along several habitat niche dimensions[11,12], as well as the food axis[11]. Hanski and Ranta[14] proposed that the distributions of *Daphnia* in rockpools could be explained in terms of a colonization–extinction model, in which interspecific competition was assumed to influence extinction- and colonization rates. Such metapopulation models, where a metapopulation is defined as a regional population consisting of a number of patchily distributed local populations, have begun to play an important part in basic as well as applied ecology[16,17], but there is little experimental or field evidence to support them.

The field experiment used the three *Daphnia* species mentioned[10,11]. It was conducted during 1983–86 and 1984–87 in artificial rockpools of four different sizes, namely 4-, 12-, 50- and 300-litre plastic bowls, placed outdoors and filled with freshwater. Experiments were run with the three-species combination, the three two-species combinations, and several one-species controls (Tables 1 and 2). Probably about sixteen, but at the very least eight, *Daphnia* generations occurred during the four-year experimental period[11]. In addition, species occurrences and environmental conditions were studied in more than 400 natural rockpools in three areas along the Swedish coast for five years (ref. 11; Tables 3 and 4).

Extinction rates (population^{-1}. yr^{-1}) were always higher in the three-species- than in the two-species experiments, and no extinctions occurred in the one-species controls (Table 1). Extinction rates were also greater the smaller the artificial pools (Table 1). Analysis of the results of all the 4-litre experiments, and those experiments that persisted until the end of the fourth

TABLE 1 Probabilities of extinction, average mean densities and average coefficients of variation in density of *Daphnia* populations in artificial rockpools

Volume (litres)	Extinction probability (per population per year) ± s.d. one-species experiments	two-species experiments	three-species experiments	Average mean population density (individuals per litre) ± s.d. Persisting populations	Extinct populations	Average coefficient of variation in density ± s.d. Persisting populations	Extinct populations
4	0 (—) (n = 6)	0.21 (±0.078) (n = 28)	0.28 (±0.11) (n = 18)	—	—	—	—
12	0 (—) (n = 3)	0.028 (±0.019) (n = 22)	0.20 (±0.10) (n = 12)	125* (±97.9) (n = 22)	5.4* (±3.66) (n = 5)	131 (±38.3) (n = 22)	122 (±40.8) (n = 5)
50	0 (—) (n = 4)	0 (—) (n = 26)	0.10 (±0.047) (n = 15)	54.1* (±30.9) (n = 26)	10.6* (±10.4) (n = 4)	143 (±37.1) (n = 26)	188 (±64.7) (n = 4)
300	—	0 (—) (n = 2)	0.030 (±0.029) (n = 9)	32.3 (±16.6) (n = 10)	5.6 (—) (n = 1)	159 (±38.5) (n = 10)	137 (—) (n = 1)

Experiments were performed during 1983–86 or 1984–87, except 4-l experiments, which lasted for one summer (1983 or 1984). Four (±1) replicates of each two-species combination and the three-species combination were run, except for 4-l pools where six three-species experiments were run, and for 300-l pools where only one two-species experiment was run. One-species controls, with at least one for each species, were run in 4-, 12- and 50-l pools. The experiments were inoculated with a natural pond phytoplankton assemblage in the middle of May the year of starting, and about two weeks later *Daphnia* from natural rockpools were introduced[11]. *Daphnia* survived winters as resting eggs. The results are based on samples from three to four sampling dates per year spaced evenly from May to autumn. Only established populations were used when calculating extinction probabilities. An extinction was judged to have occurred if an established species present in one year was absent in the following years. The 4-l pools were sampled and analysed in their entirety (including counts of resting eggs) at the end of the summer. Extinction probabilities were calculated on a population basis, regardless of species. The mean population densities and coefficients of variation in density for each population in each two- and three-species experiment were used for calculating averages in persisting and extinct populations. Some vessels broke during winters, and those present in only the first year were excluded from these analyses. For extinct populations, only values of population densities before extinction had occurred were used. Coefficients of variation were corrected for unequal sample sizes. See ref. 11 for further information and data. n, Initial number of populations; asterisk indicates significant difference between persisting and extinct populations (Mann–Whitney U Test, $P < 0.01$).

TABLE 2 Number of extinctions of *Daphnia* species in artificial rockpools after a 4-year experimental period

Volume (litres)	Number of extinctions of *Daphnia* in different combinations						
	M	*P*	*L*	*M*+*P*	*M*+*L*	*P*+*L*	*M*+*P*+*L*
4	0 (n=3)	0 (n=2)	0 (n=1)	*M* 0 (n=4) *P* 2 (n=4)	*M* 0 (n=5) *L* 2 (n=5)	*P* 0 (n=5) *L* 2 (n=5)	*M* 0 (n=6) *P* 3 (n=6) *L* 2 (n=6)
12	0 (n=1)	0 (n=1)	0 (n=1)	0 (n=2)	0 (n=3)	*P* 0 (n=4) *L* 2 (n=4)	*M* 2 (n=3) *P* 0 (n=3) *L* 1 (n=3)
50	0 (n=1)	0 (n=2)	0 (n=1)	0 (n=1)	0 (n=4)	0 (n=3)	*M* 0 (n=3) *P* 3 (n=3) *L* 1 (n=3)
300	—	—	—	—	0 (n=1)	—	*M* 0 (n=3) *P* 1 (n=3) *L* 0 (n=3)

n, Number of populations of each species in pools persisting for four years (in 4 litres for one year). Values of n differ between species combinations because several bowls broke during winters. *Daphnia magna* (*M*), *D. pulex* (*P*) and *D. longispina* (*L*). In one 4-l and one 50-l experiment, *D. pulex* and *D. longispina* went extinct at about the same time. There were significantly more extinctions in three-species than in two-species experiments (P=0.00484). Because of low values of n and a large number of pools with no extinctions, conventional statistical tests were not possible. Instead, the numbers of extinctions in two- and three-species experiments at the end of the experimental period in each of the four volumes were analysed using separate Fisher's exact tests. A procedure was then attempted that corresponded to Fisher's combined probability test[23], but because the exact tests had discrete, not continuous, probability distributions, this test was not valid[24,25]. The product of the exact probabilities of the observed or more extreme results in each volume was taken, and the probability of obtaining the observed product or a smaller one, out of the 720 possible products, was calculated using a computer (when the observed number of extinctions in each volume was kept, there were 12, 6, 5 and 2 possible probabilities in 4-, 12-, 50- and 300-l experiments, respectively). In the present case, the observed P-values in each volume are 0.4398 (4 l), 0.1889 (12 l), 0.00996 (50 l) and 0.8182 (300 l), and the probabilitity of obtaining the product 0.0006768 or smaller is 0.004839 (all probabilities are one-tailed).

year in the larger volumes showed that there were significantly more extinctions in three-species experiments (13 of 45 populations) than in two-species experiments (8 of 64 populations) (P = 0.00484; Table 2). Of the 21 extinctions observed overall, the large species *D. magna* went extinct fewer times than the smaller *D. pulex* and *D. longispina* (2, 9 and 10 extinctions respectively; Table 2; pooled data, G-test: G = 6.97, 2 degrees of freedom, $P < 0.05$).

Detailed analyses of population dynamics and reproduction demonstrated that the three species used common food resources, and that this led to severely depressed food levels, strongly reduced reproductive rates, and intense interspecific competition during substantial parts of each year[11]. Because predators were excluded by insect nets, and the experimental vessels never dried up, the observed extinctions most probably were attributable to interspecific competition. The populations that subsequently went extinct generally had lower mean densities than persisting ones, but the coefficients of variation in density did not differ between extinct and persisting populations (Table 1). Thus small population sizes in conjunction with intense interspecific competition apparently made populations in these experiments vulnerable to extinction.

All three species have not been observed together in the same pool either in natural rockpools in the three Swedish areas[11], or in southwestern Finland[12,15] (Tables 3 and 4). Extinction rates in two-species pools in all the four areas were higher than in one-species pools; on average 18 and 11% of the populations went extinct per year, respectively (Table 3; n-weighted averages)[11,15]. In natural rockpools, there were no significant consistent differences in extinction rates between the three species (Table 4).

Thus, interspecific competition seems to influence the distribution and metapopulation dynamics of *Daphnia* species in the field[11,14]. In particular, it may contribute to the scarcity of three-species pools by reducing the longevity of three-species combinations. Several observations, however, suggest that interspecific competition is only one of several factors causing natural extinctions in rockpools. First, no extinctions occurred in the larger (50- and 300-litre) two-species experiments (Tables 1 and 2). Second, many population turnovers in natural rockpools could be attributed to variation in abiotic factors such as salinity[11]. Third, predators such as newts, fish and water bugs, if present, can easily drive *Daphnia* populations to extinction[11,18,19].

The results presented here indicate that interspecific competition may limit species richness in the rockpool metapopulation system. Two *Daphnia* species seem to be able to coexist in single pools for extended periods of time despite no, or little, possibility for niche differentiation (ref. 11, and personal observations). The three species coexist regionally, but not all together in single pools, in several areas in Scandinavia[11-15], a finding that is

TABLE 3 Extinction rates in natural rockpools with one- and two *Daphnia* species in four areas of Scandinavia

Area	Extinction rate (probability of extinction per population per year) ± s.d.	
	One species present	Two species present
Flatholmen	0.13 (±0.037) (n=82)	0.15 (±0.046) (n=58)
Mönster	0.12 (±0.038) (n=74)	0.42 (±0.14) (n=12)
Ängskär	0.097 (±0.025) (n=143)	0.17 (±0.051) (n=54)
Tvärminne	0.11 (±0.028) (n=123)	0.16 (±0.052) (n=50)

n, Total number of possible extinction events during the study period. There were significantly more extinctions in rockpools with two species present than in one-species pools (Fisher's combined probability test[23] using probabilities from G-tests for each area separately: P=18.25, eight degrees of freedom, $P < 0.02$). The areas are Flatholmen island and Mönster peninsula on the Swedish west coast[11], Ängskär islands in the Stockholm archipelago on the Swedish east coast[11], and islands near Tvärminne in south-western Finland[15]. Extinction rates on Mönster and Ängskär were calculated using data on species occurrences in rockpools during 1982–86, and on Flatholmen using data from 1982–84 and 1986–87. An extinction was judged to have occurred if a species present in one year was absent from the samples in two successive years. Extinction rates were calculated as the sum of the number of observed extinctions in populations present in each of the years 1982, 1983 and 1984, divided by the total number of possible extinctions during these years. See also ref. 11. In the Tvärminne area, extinction rates were calculated as the number of populations present in 1983 that were extinct by 1985, divided by the number of populations present in 1983 (Tables 2 and 6 in ref. 15 were used).

TABLE 4 Number of extinctions of *Daphnia* species in natural rockpools in three areas in Sweden

Area	Number of extinctions of *Daphnia* in different species combinations						
	M	*P*	*L*	*M*+*P*	*M*+*L*	*P*+*L*	*M*+*P*+*L*
Flatholmen	12 (n=60)	0 (n=5)	3 (n=17)	0 (n=0)	*M* 7 (n=29) *L* 5 (n=29)	0 (n=0)	0 (n=0)
Mönster	6 (n=61)	3 (n=13)	—	*M* 2 (n=6) *P* 3 (n=6)	—	—	—
Ängskär	6 (n=46)	4 (n=75)	4 (n=22)	*M* 1 (n=16) *P* 3 (n=16)	*M* 2 (n=10) *L* 2 (n=10)	*P* 0 (n=1) *L* 1 (n=1)	0 (n=0)

n, Number of possible extinction events in each species. *Daphnia magna* (*M*), *D. pulex* (*P*) and *D. longispina* (*L*). No *D. longispina* were found in the Mönster area. There were no significant differences in extinction rates between the three species (G-tests, $P > 0.05$).

consistent with immigration–extinction models for coexistence of similar competitors in patchy habitats[14,16,20]. The field experiments demonstrated, however, that extinction rates increased with an increasing number of species initially present; this indicates that regional coexistence of a large number of cladoceran species in this system is unlikely.

It has been debated whether interspecific competition is an important factor determining species distributions and limiting species richness in natural communities[1–7,21,22]. The combination of experimental and observational evidence presented here shows that interspecific competition can be important in these respects, and that it may be essential to incorporate the process in studies of metapopulation systems. □

Received 20 February; accepted 20 July 1989.

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ACKNOWLEDGEMENTS. I thank L. Gustafsson, J. H. Lawton, A. Pape Møller, S. G. Nilsson, S. Ulfstrand, and G. I. Ågren for comments, and L. Norell for statistical advice. The study was supported by grants from the Swedish Natural Science Research Council and by Sven and Lilly Lawski's Foundation for Natural Science Research.

Noradrenaline and serotonin selectively modulate thalamic burst firing by enhancing a hyperpolarization-activated cation current

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NEURONS in many regions of the mammalian nervous system generate action potentials in two distinct modes: rhythmic oscillations in which spikes cluster together in a cyclical manner, and single spike firing in which action potentials occur relatively independently of one another[1,2]. Which mode of action potential generation a neuron displays often varies with the behavioural state of the animal[2,3]. For example, the shift from slow-wave sleep to waking and attentiveness is associated with a change in thalamic neurons from rhythmic burst firing to repetitive single spike activity, and a greatly increased responsiveness to excitatory synaptic inputs[1–3]. This marked change in firing pattern and excitability is controlled in part by ascending noradrenergic and serotonergic inputs from the brainstem[4–11], although the cellular mechanisms of this effect have remained largely unknown. Here we report that noradrenaline and serotonin enhance a mixed Na^+/K^+ current which is activated by hyperpolarization (I_h) and that this enhancement may be mediated by increases in intracellular concentration of cyclic AMP. This novel action of noradrenaline and serotonin reduces the ability of thalamic neurons to generate rhythmic burst firing and promotes a state of excitability that is conducive to the thalamocortical synaptic processing associated with cognition.

Application of noradrenaline (NA; 0.5 mM) or serotonin (5-HT; 0.3 mM) to guinea-pig (n = 125) or cat (n = 6) dorsal lateral and medial geniculate neurons at resting membrane potential (−60 to −68 mV), resulted in a small depolarization associated with an increase in apparent membrane conductance (Fig. 1*a*, *b*). The current-versus-voltage (I–V) relationships show that both the NA- and 5-HT effects were highly voltage-dependent and appeared as an inward current at membrane potentials negative to ~ −60 mV (Fig. 1*c*, *d*). The membrane potential at which the response first became apparent was similar for NA (−65 ± 7 mV, mean ± s.d., n = 18) and 5-HT (−69 ± 6 mV, n = 7). This effect of NA and 5-HT was a direct postsynaptic response because it persisted after blocking synaptic transmission by exposing the tissue slices to a low-Ca^{2+} (0.5 mM)- and high Mn^{2+} (4 mM)-containing medium (n = 3). In contrast to the voltage-dependent responses to NA and 5-HT (Fig. 1*c*, *d*), increases in membrane Cl^- conductance, elicited by the $GABA_A$ (γ-aminobutyric acid) agonist muscimol (n = 11), and K^+ conductance, elicited by the $GABA_B$ agonist baclofen (n = 7)[12], displayed reversal potentials and were less voltage dependent (Fig. 1*e*, *f*).

The NA-induced response appeared to be mediated by β-adrenergic receptors because prazosin (2 μM) and yohimbine (1 μM) were routinely added to the perfusion medium to block

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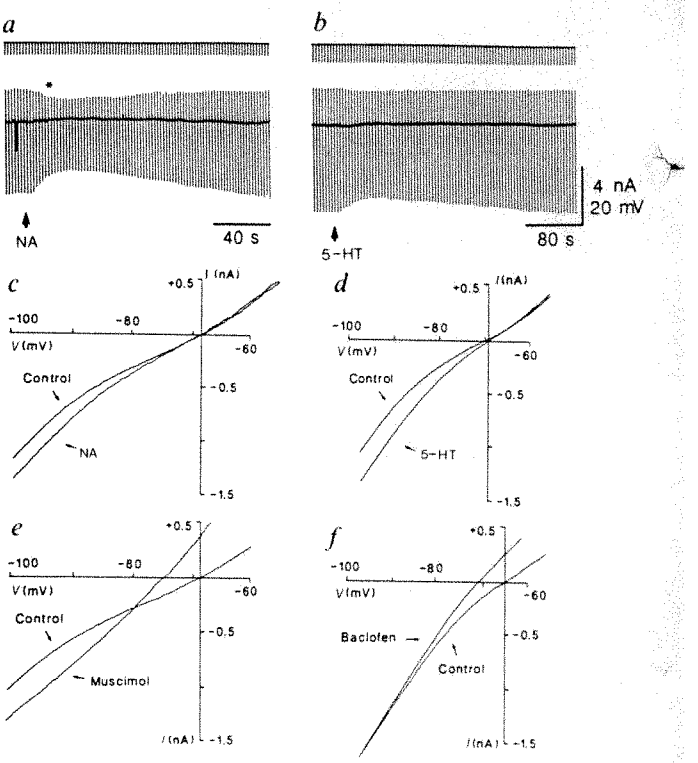
FIG. 1 Responses of thalamic neurons to NA and 5-HT. *a*, Application of NA to a guinea pig dorsal lateral-geniculate neuron during the injection of hyperpolarizing constant current pulses (top trace) results in a small depolarization and a large increase in apparent membrane conductance (bottom trace). Upward going lines in this and all subsequent voltage traces represent rebound Ca^{2+} spikes (indicated by asterisk; spikes are truncated by slow frequency response of chart recorder). *b*, Application of 5-HT to a cat medial geniculate neuron has a similar effect. *c*, *d*, The *I–V* relationships obtained in voltage clamp before and after application of NA and 5-HT show a progressive increase in inward current at membrane potentials negative to about −60 mV. *e*, In contrast to NA and 5-HT responses, increasing membrane Cl^- conductance by activating $GABA_A$ receptors with muscimol (100 μM) shows no such voltage sensitivity. *f*, Increasing membrane K^+ conductance by activating $GABA_B$ receptors with baclofen (100 μM) results in an outward current that reverses at about −105 mV. Data in *c–f* are obtained from the same guinea pig dorsal lateral-geniculate neuron. The small depolarizations associated with responses to NA and 5-HT in *a*, *b* were smaller than usual.

METHODS. Guinea pigs or cats (two) were deeply anaesthetized with sodium pentobarbital and killed by decapitation, as described previously[28–30]. The medial and lateral geniculate nuclei were rapidly dissected free and were sectioned coronally on a vibratome as 400-μm slices. Slices were maintained in an interface chamber at 36 ± 1 °C and perfused with a solution containing (mM): NaCl (126), KCl (2.5), $MgSO_4$ (2), $NaHCO_3$ (26), NaH_2PO_4 (1.25), $CaCl_2$ (2), glucose (10); saturated with 95% O_2, 5% CO_2 to final *p*H 7.4. Changing extracellular ionic concentrations was achieved by the following substitutions: choline chloride for NaCl; KCl for NaCl; and sodium isethionate for NaCl, potassium acetate for KCl. When Mn^{2+} was added to the bathing medium, sulphate and phosphate were omitted to prevent precipitation. Only neurons having stable membrane potentials negative to −55 mV and overshooting action potentials were included for analysis. Unless stated otherwise, neuroactive substances were dissolved in the bathing medium and applied in volumes of 5–20 pl locally to the surface of the slice near the recording site through a broken intracellular micropipette (2–5-μm tip diameter) by pulses of pressure. Intracellular recording microelectrodes were filled with 4 M potassium acetate and had a final resistance of 25–50 MΩ. Single electrode voltage clamp was achieved with an Axoclamp-2A amplifier (Axon Instruments) coupled to an IBM AT computer operating pClamp software (Axon Instruments). Headstage output was continuously monitored and sampling frequencies were between 3.5 and 5.5 KHz.



Amplifier gain was typically ~1 nA mV^{-1}. *I–V* relationships were obtained by applying a hyperpolarizing voltage ramp from ~ −50 to −100 mV. Thalamic neurons respond to NA with large depolarizations through a decrease in K^+ conductance mediated by α_1-receptors[29]. To isolate the effects of NA through β-receptors for the present study, prazosin (2 μM) and yohimbine (1 μM) were routinely added to the bathing medium. Responses to 5-HT and isoprenaline were identical in either normal or prazosin–yohimbine containing medium.

α-receptors, and NA-induced responses were mimicked by the β-adrenergic-specific agonist isoprenaline (50–250 μM, $n = 12$). Application of the β-adrenergic antagonists propranolol (local application, 100 μM in micropipette, $n = 2$) and atenolol (15 μM in the bath, $n = 2$) reversibly blocked responses to NA but not to 5-HT. By contrast, local (10–100 μM, $n = 4$) or bath (1–5 μM, $n = 4$) application of the 5-HT_1 and 5-HT_2 antagonist methysergide blocked the response to serotonin without affecting the response to NA. The 5-HT_{1A} agonist ipsapirone (0.2 mM), the partial agonist 8-OHDPAT (8-hydroxy-dipropylaminotetralin) (0.1–1 mM, $n = 4$), the 5-HT_2 antagonist ritanserin (5 μM in the bath, $n = 4$), all had no effect on dorsal lateral geniculate neurons or on the response of these neurons to 5-HT. This indicates that the 5-HT_{1A} and 5-HT_2 receptor subtypes are not involved in the response to 5-HT. This finding is in agreement with the non-5-HT_{1A}, non-5-HT_2 pharmacological profile of serotonin binding-sites in the medial and lateral geniculate nuclei[13,14].

The marked similarity between the NA- and 5-HT responses suggests that they may have been affecting the same current. Indeed, we found that a maximal application of 5-HT greatly reduced or blocked the response to NA in voltage clamp ($n = 4$), a phenomenon known as non-additivity, indicative of convergence of neurotransmitter action[15].

Because the NA- and 5-HT-induced responses were strongly voltage dependent, we hypothesized that NA and 5-HT could

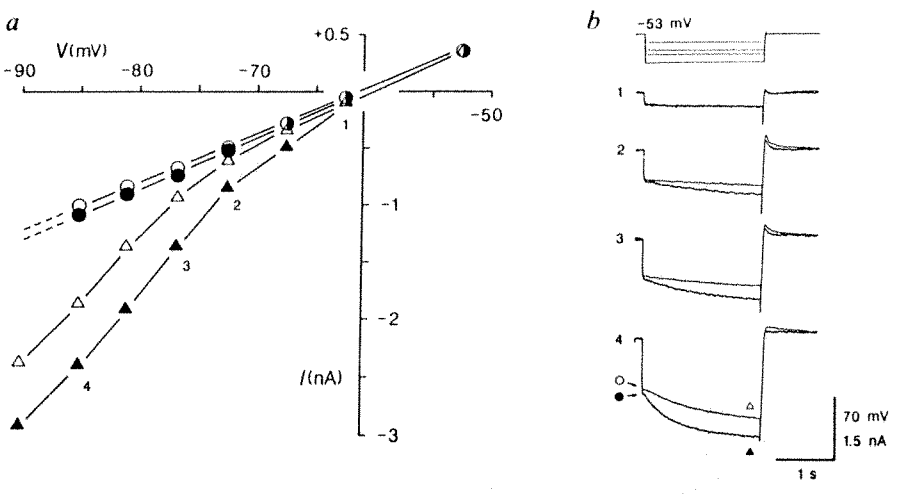


FIG. 2 Noradrenaline enhances a current activated by hyperpolarization. *a*, Graph of the instantaneous (circles) and steady-state (triangles) currents in response to a hyperpolarizing voltage step before (open symbols; control) and after (filled symbols) application of NA. Original traces are shown in *b*. Application of NA results in a marked enhancement of the inward current activated by hyperpolarization, with only a small increase in instantaneous conductance.

have been enhancing a current activated by hyperpolarization. Neurons in other brain regions, and cells in the heart, possess inward currents that are activated by hyperpolarization and have been variously termed I_Q, I_f, I_{AR}, I_h and $I_{Cl(v)}$[16-22]. To test for the presence of such currents in thalamic neurons, we performed incrementing voltage steps from a holding potential of −50 mV to between −60 and −100 mV. Hyperpolarization negative to −60 mV activated a slowly developing inward current, which we term I_h. The amplitude and rate of activation of I_h increased with membrane hyperpolarization (Fig. 2). The time course of activation of I_h was well fitted (coefficient of correlation, $r > 0.99$) to a single exponential function with a time constant of between 0.2 (−100 mV) and 2.0 (−70 mV) seconds. Application of NA or 5-HT resulted in a substantial enhancement of I_h, with little, if any, change in instantaneous membrane conductance (Fig. 2, $n = 11$). Interestingly, the activation time course of I_h was significantly faster after application of NA or 5-HT (time constant = 1,092 ± 262 ms pre-application; 712 ± 166 ms post-application; $t = 4.8$ $P < 0.01$; voltage step from −50 to −80 mV; $n = 5$), as has been reported for noradrenergic enhancement of I_f in the heart[23-25].

Stimulation of β-adrenergic receptors in many regions of the nervous system leads to increases in the intracellular concentration of cAMP (ref. 26). In the heart, it is this increase in intracellular cAMP that is thought to couple β-adrenergic receptors to increases in the hyperpolarization activated 'pacemaker' current I_f (ref. 25). In thalamic neurons we found that local application of the membrane-permeable cAMP analogue 8-bromo-cAMP (500 μM, $n = 5$), the adenylyl cyclase stimulant forskolin (25 μM, $n = 4$), or the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX, 300 μM, $n = 3$) all resulted in a marked enhancement of I_h with little or no change in instantaneous 'leak' conductance (data not shown). Local application of the forskolin analogue 1,9-dideoxy-forskolin (50–100 μM, $n = 4$), which does not activate adenylyl cyclase, had no effect. These results suggest that the β-adrenergic and/or serotonergic enhancement of I_h may be mediated by an increase in intracellular concentration of cAMP.

The ionic nature underlying I_h and the NA- and 5-HT-induced response was investigated by changing the concentration gradient of Na^+, K^+, and Cl^- across the neuronal membrane (Fig. 3). Reducing the extracellular concentration of Na^+ ($[Na^+]_o$) from 153 mM to 26 mM resulted in a parallel shift on the voltage axis of the I–V curve for I_h and the NA response by −14 mV (±3 mV, $n = 4$) (Fig. 3*a*). Similarly, shifting $[Na^+]_o$ from 26 mM to 153 mM shifted the I–V curve by +19 mV (±7 mV, $n = 3$). Increasing $[K^+]_o$ from 2.5 to 7.5 mM shifted I_h and the NA-induced response by +11 mV (±1 mV, $n = 6$) (Fig. 3*b*). Returning $[K^+]_o$ to normal reinstated the original response in one cell. These observed changes correspond to a shift of ~21 and 25 mV per tenfold change in $[Na^+]_o$ and $[K^+]_o$, respectively.

Reducing $[Cl^-]_o$ from 132.5 to 4 mM did not affect the response to NA (Fig. 3*c*, $n = 5$), but did result in a large shift (up to +70 mV) in the reversal potential of responses to the $GABA_A$ agonist muscimol (Fig. 3*c*). Similarly, intracellular injection of NO_3^-, which readily passes through Cl^- channels but does not support outward Cl^- transport[27], resulted in large

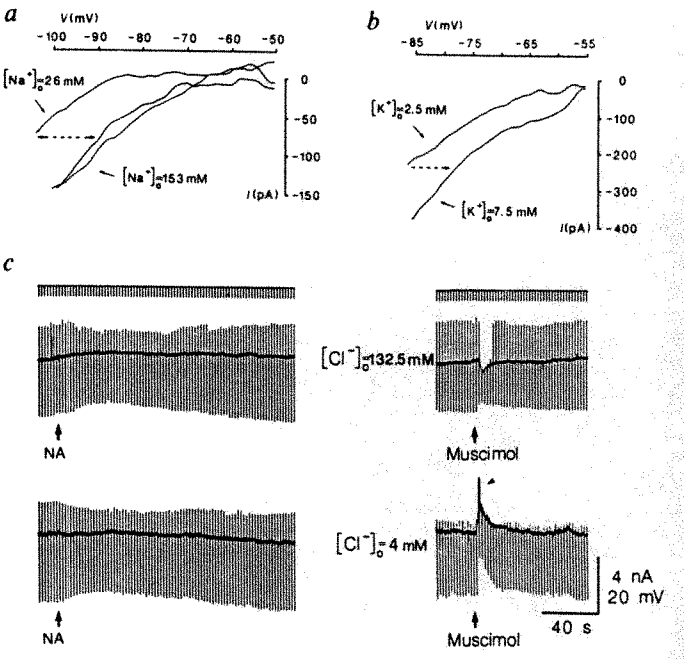


FIG. 3 Effects of changing extracellular Na^+, K^+ and Cl^- concentrations on the response to NA. *a*, Plot of the current activated by NA versus membrane potential in normal $[Na^+]_o$ (153 mM), after washing in low $[Na^+]_o$ (26 mM), and recovery back to normal $[Na^+]_o$. The NA-response I–V curve is found to shift by ~16 mV. *b*, Changing $[K^+]_o$ in another neuron from 2.5 to 7.5 mM shifts the curve by ~ +12 mV. The NA-response I–V curves were obtained by subtracting the I–V plots with NA from those obtained before NA addition. *c*, Reducing $[Cl^-]_o$ from 132.5 to 4 mM shifts the response to the $GABA_A$ agonist muscimol from a small hyperpolarization to a large depolarization, which elicits action potentials (arrow head). By contrast, reducing $[Cl^-]_o$ has no effect on the response to NA.

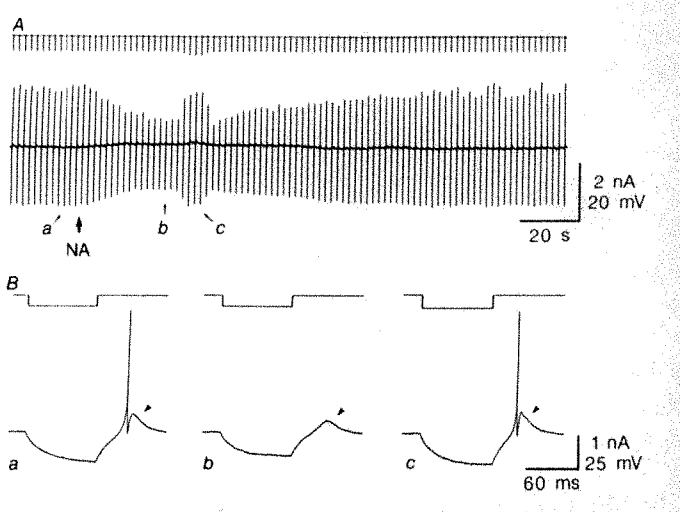


FIG. 4 Effect of NA on the response of a lateral geniculate neuron to hyperpolarizing current pulses. Intracellular injection of hyperpolarizing current pulses typically results in a rebound Ca^{2+}-mediated spike[32,33] (*B*, arrow heads) that can activate Na^+/K^+-mediated action potentials. Application of NA substantially decreases the hyperpolarizing response and subsequently reduces the amplitude of the rebound Ca^{2+}-mediated spike (*B*, compare *a* and *b*). Increasing the amplitude of the current pulse so that the hyperpolarization matches that before NA addition reinstates the full Ca^{2+}-mediated spike (*B*, *c*). Traces expanded in *B* are as indicated in *A*. The ability of NA to reduce all components of the hyperpolarizing pulse results from an increase in the amount of I_h active at resting membrane potential as well as the amount that is activated by the hyperpolarizing pulse (data not shown).

depolarizing responses to muscimol, but normal responses to NA ($n = 6$, not shown).

These results demonstrate that NA and 5-HT enhance a hyperpolarization-activated current (I_h) that is carried by Na^+ and K^+ ions. In further support of this, we found that both I_h and the response to NA and 5-HT were blocked by introducing Cs^+(2 mM) but not Ba^{2+} (0.5 mM) into the bathing medium ($n = 9$).

Rhythmic burst firing in thalamic relay neurons during periods of electroencephalographic (EEG) synchronization (such as slow-wave sleep) results from the generation of low threshold Ca^{2+} spikes, which appear as rebound responses arising from rhythmic inhibitory postsynaptic potentials (i.p.s.p.s) from the nucleus reticularis[1,2]. To test a possible effect of NA or 5-HT on rhythmic burst firing, we injected short duration (40–80 ms, 0.5–4.0 Hz) hyperpolarizing current pulses to mimic rhythmic i.p.s.p.s. Application of NA or 5-HT substantially decreased both the voltage deviation caused by the hyperpolarizing pulse as well as the rebound Ca^{2+} spike (Fig. 4). The latter was a secondary effect, as increasing the pulse size reinstated the full response (Fig. 4). By contrast, NA had only very weak facilatatory effects on the response of these same cells to depolarizing current pulses and no consistent effect on the firing rate of neurons that were manually depolarized above single-spike firing threshold (about −55 mV, data not shown).

The results of this study reveal a potent and novel action for NA and 5-HT in the central nervous system. In the thalamus, the NA- and 5-HT-induced increase in I_h results in a selective dampening of neuronal responsiveness to large hyperpolarizing inputs, with little effect on phasic or tonic depolarizations. Together with the other actions of acetylcholine and NA, this effect may be responsible for the marked increase in the efficacy of transfer of information through the thalamus during periods of increased arousal and attentiveness[28–31]. The coexistence of I_h or analogous currents, and a serotonergic or noradrenergic projection at all levels of the nervous system, implies that the present findings may have widespread implications for the neurotransmitter control of neuronal excitability[16–22]. □

Received 10 March; accepted 7 July 1989.

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ACKNOWLEDGEMENTS. This work was supported by the Jacobs Javits Center for Neuroscience, the NIH, a fellowship from the Klingenstein Foundation (D.A.M.) and a fellowship from the Deutsche Forschungemeinschaft (H.C.P.).

Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes

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In heart cells, cyclic AMP-dependent protein kinase (PKA) regulates calcium-[1–4] and potassium-ion current[1,2,5,6] by phosphorylating the ion channels or closely associated regulatory proteins. We report here that isoprenaline induced large chloride-ion currents in voltage-clamped, internally-dialysed myocytes from guinea-pig ventricles. The Cl^- current could be activated by intracellular dialysis with cAMP or the catalytic subunit of PKA, indicating regulation by phosphorylation. In approximately symmetrical solutions of high Cl^- concentration, the macroscopic cardiac Cl^- current showed little rectification, unlike the single-channel current in PKA-regulated Cl^- channels of airway epithelial cells[7]. But, like epithelial Cl^--channel currents, the cardiac Cl^- current was sensitive to the distilbene, 4,4′-dinitrostilbene-2,2′-disulphonic acid (DNDS)[8]. In the absence of kinase activation, cardiac sarcolemmal Cl^- conductance was negligible. During β-adrenergic stimulation of the heart, this novel Cl^- conductance should accelerate action-potential repolarization and so protect impulse propagation in the face of the possibly arrhythmogenic increases in heart rate and in calcium entry into the cells.

Application of 100 nM isoprenaline to a myocyte bathed in 146 mM Cl^- solution, and equilibrated with Na^+-, K^+-, and Ca^{2+}-free pipette solution containing 135 mM Cl^-, caused little change in whole-cell current at the 0 mV holding potential, which was chosen to inactivate Na^+ and Ca^{2+} channels (Fig. 1*A*). Voltage pulses of 80 ms to membrane potentials between −100 mV and +100 mV revealed, however, large isoprenaline-activated currents (Fig. 1*B*, $b - a$), whose amplitude varied approximately linearly with voltage (Fig. 1*D*), and which reversed near 0 mV, close to the Cl^- equilibrium potential (E_{Cl}, estimated to be −2 mV under these conditions). That the isoprenaline-induced current was indeed an anion current carried by Cl^-, and not a cation current carried, for example, by extracellular Na^+ and intracellular Cs^+ through a non-specific pathway, is shown by the virtual abolition of the inward current at negative potentials (Fig. 1*B*, *C*, *D*), and by the concomitant appearance of an isoprenaline-induced outward shift of the holding current at 0 mV (Fig. 1*A*), after exchange of the Cl^--containing solution within the pipette for a Cl^--free solution, containing instead 90 mM aspartate and 20 mM methanesulphonate.

Further evidence that the isoprenaline-activated current was carried by Cl^- is presented in Fig. 2*A*, which shows that, at positive potentials, the large outward current elicited by isoprenaline in a cell bathed in 146 mM Cl^- solution, was virtually absent after all but 1 mM extracellular Cl^- was replaced by isethionate ions. Because the intracellular Cl^- concentration ($[Cl^-]_i$) was kept constant at 20 mM, this drastic reduction of extracellular Cl^- concentration ($[Cl^-]_o$) caused the reversal potential for the isoprenaline-induced current (and E_{Cl}) to shift from a negative potential to a positive potential (Fig. 2*Ab*). This, in turn, caused the isoprenaline-induced shift of the holding current at 0 mV to reverse from *outward* to *inward* (Fig. 2*Aa*). The record of whole-cell current in Fig. 2*Ba* shows that

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the pronounced outward current activated by isoprenaline at 146 mM $[Cl^-]_o$ could be abolished by subsequent application of 0.1 mM DNDS, one of the disulphonic stilbene derivatives which are potent blockers of anion fluxes in a variety of cell types[8-11]. The whole-cell current-voltage (I-V) relationships in Fig. 2*Bb* demonstrate that, over the entire voltage range examined, membrane current in the combined presence of isoprenaline and DNDS was almost the same as that in the absence of both.

Isoprenaline (100 nM) activated large steady Cl^- currents (>0.5 pA pF^{-1} at +100 mV) in 33 of the 42 cells tested at 146 mM $[Cl^-]_o$. The lack of any change in holding current (at 0 mV) on removing either intracellular Cl^- (Fig. 1*A*) or extracellular Cl^- (Fig. 2*Aa*) in the absence of isoprenaline, however, and the identity of whole-cell I-V relationships with and without internal Cl^- (Fig. 1*C*) or external Cl^- (not illustrated), indicates that membrane Cl^- conductance is negligible unless β-adrenoceptors are activated.

Like the unitary Cl^--channel currents in airway epithelial cells[12-15] and lymphocytes[16], these Cl^- currents elicited by isoprenaline in myocytes seem to be regulated by PKA. Thus, large outward currents, with voltage dependence similar to those activated by isoprenaline (Fig. 3*Ab*, *Bb*), were seen if myocytes exposed to 146 mM $[Cl^-]_o$ were internally dialysed with either cAMP (Fig. 3A) or the catalytic subunit of PKA (Fig. 3*B*). Furthermore, the strong outward current at positive potentials induced by putting cAMP in the pipette was diminished, in another cell, by ~80% with 0.01 mM external DNDS, and by >90% with 0.1 mM DNDS (not illustrated), and three-quarters of that activated by the PKA subunit was abolished on lowering $[Cl^-]_o$ from 146 mM to 1 mM (Fig. 3*Bb*). With the wide-tipped, low resistance (1-5 MΩ) pipettes used in these experiments, the Cl^- conductance was usually activated within seconds of switching to a pipette solution containing 1 mM cAMP (for example, see Fig. 3*Aa*), but took more than 15 min to appear after introducing the PKA catalytic subunit to the pipette tip in the experiment of Fig. 3*Ba*. Although small molecules like cAMP diffuse readily into the cell from the pipette[1,17], the relatively large protein subunit (relative molecular mass M_r of 40,000) can be expected to take many minutes to half equilibrate with the cell interior[1].

The cAMP-dependent Cl^- currents described here do not seem to require intracellular Ca^{2+} (refs 7, 13, 18), because Ca^{2+} currents were inactivated (Fig. 1*B*) by the 0 mV holding potential, and pipette solutions contained 10 mM (Figs 1, 2*B* and 3*A*) or 50 mM (Figs 2*A* and 3*B*) EGTA and no added Ca^{2+}.

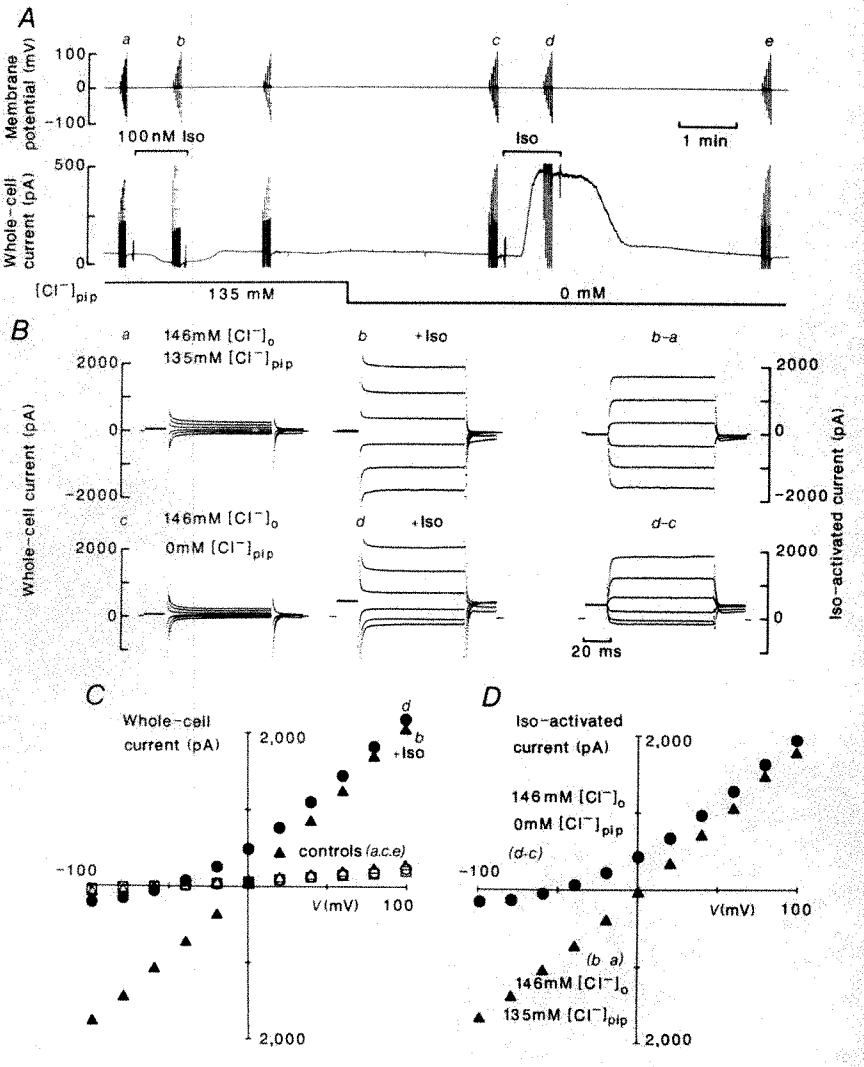

FIG. 1 Voltage dependence and sensitivity to $[Cl^-]_i$ of isoprenaline-induced current. *A*, Chart recording of membrane potential (upper trace) and whole-cell current (lower trace). Upper trace, the groups of vertical lines indicate acquisition of I-V data during 80-ms voltage pulses from the holding potential, 0 mV, to potentials between −100 and +100 mV. Lower trace, the bars mark exposure to 100 nM isoprenaline (Iso). The lower line indicates the change in pipette chloride concentration ($[Cl^-]_{pip}$) from 135 to 0 mM (replaced by aspartate and methanesulphonate). Extracellular chloride concentration was constant at 146 mM. *B*, Superimposed records of whole-cell currents for pulses to +100, +60, +20, −20, −60, and −100 mV, recorded in the absence (*a*, *c*) or presence (*b*, *d*) of isoprenaline, with either 135 mM $[Cl^-]_{pip}$ (upper traces) or 0 mM $[Cl^-]_{pip}$ (lower traces). Isoprenaline-activated currents (*b* − *a*, *d* − *c*) were obtained by subtracting currents recorded in the absence of isoprenaline from corresponding currents recorded during the exposure to isoprenaline. *C*, Whole-cell I-V relationships from the data in *A* and *B*, in the absence (△, ○, □) or presence (▲, ●) of isoprenaline, with either 135 mM Cl^--containing (△, ▲) or Cl^--free (○, ●, □) pipette solution: steady-state current levels were measured near the end of each pulse. *D*, Steady-state levels of isoprenaline-activated currents determined from data represented in *B*, plotted against membrane potential; 135 mM $[Cl^-]_{pip}$ (▲, *b* − *a*) or 0 mM $[Cl^-]_{pip}$ (●, *d* − *c*). Total cell capacitance = 138 pF; initial pipette resistance = 3.3 MΩ.

METHODS. Myocytes were isolated from guinea-pig ventricles with collagenase, and then incubated in a high K^+-concentration, low Ca^{2+}-concentration medium[25] before being superfused at 35 °C with Tyrode's solution containing (mM): NaCl (145), KCl (5.4), $CaCl_2$ (1.8), $MgCl_2$ (2.3), dextrose (5.5), HEPES/NaOH (5) (*p*H 7.4). Giga-ohm seals were obtained with wide-tipped, fire-polished, pipettes (resistance = 1-5 MΩ) filled with Tyrode's that was then exchanged[26], before rupture of the cell membrane, for a Cl^--free pipette solution containing (mM): CsOH (~145), aspartic acid (90), MgATP (10), $Tris_2$-creatine phosphate (5), tetraethylammonium-methanesulphonate (20), EGTA (10), HEPES (10) (*p*H 7.4). The $[Cl^-]_{pip}$ was raised to 135 mM by adding Cl^- and omitting aspartic acid and methanesulphonate. Voltage-clamped[27] cells were then superfused with K^+-free, low Ca^{2+}-concentration (0.5 mM $CaCl_2$) and low Mg^{2+}-concentration (0.5 mM $MgSO_4$) Tyrode's solution. The holding potential was set to 0 mV to inactivate Na^+ and Ca^{2+} channels (see Fig. 1*B*). Potassium-channel currents were minimized by omitting K^+, and including Cs^+ and tetraethylammonium in pipette solutions; Na^+/K^+ pump current was prevented by omitting Na^+ from pipette solutions and K^+ from external solutions, and Na^+/Ca^{2+} exchange current was prevented by the nominal absence of internal Na^+ and Ca^{2+}. Reduced glutathione (0.1 μM) and EDTA (20 μM) were added to solutions containing isoprenaline to slow its oxidation. The osmolality of all solutions was ~300 mosmol kg^{-1}. Current and voltage signals were low-pass filtered at 2 kHz (6-pole Bessel), digitized (12-bit resolution) on-line at 8 kHz, and stored in a microcomputer for analysis. To minimize voltage error due to a change of liquid-juncton potential on changing solution composition, pipette and bath potentials were both measured by means of miniature KCl (3 M)-filled half cells.

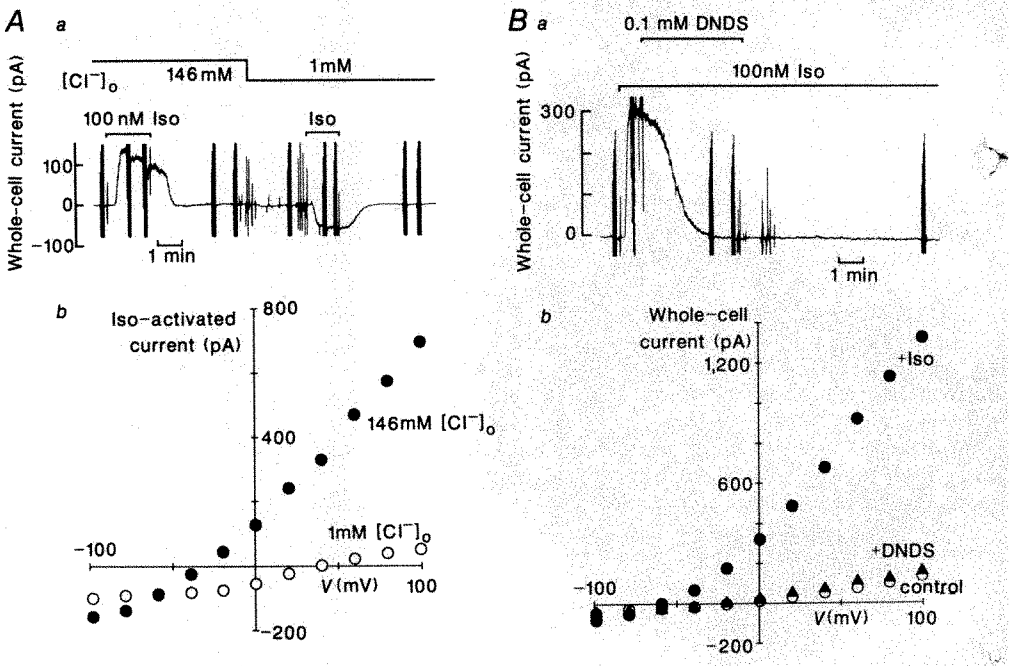

FIG. 2 Removal of isoprenaline-activated outward current by withdrawal of extracellular Cl^- (*A*) or exposure to DNDS (*B*). *Aa*, record of whole-cell current; holding potential, 0 mV. Upper line indicates reduction of $[Cl^-]_o$ from 146 to 1 mM (NaCl replaced by sodium-isethionate); $[Cl^-]_{pip}$ = 20 mM. Bars mark application of 100 nM isoprenaline. Internal (pipette) solution contained (mM): CsOH (~150), aspartic acid (16), MgATP (10), $Tris_2$-creatine phosphate (5), tetraethylammonium chloride (20), EGTA (50), HEPES (40) (*p*H 7.4). External solution was as in Fig. 1. *b*, *I–V* plots of steady isoprenaline-activated currents with 146 mM (●) or 1 mM (○) $[Cl^-]_o$. Total cell capacitance = 89 pF; initial pipette resistance = 2.9 MΩ. *Ba*, Whole-cell current; holding potential, 0 mV. Bars indicate application of 0.1 mM DNDS and/or 100 nM isoprenaline. The 0 mM $[Cl^-]_{pip}$ and 146 mM $[Cl^-]_o$ solutions were as in Fig. 1. *b*, Steady-state, whole-cell *I–V* relationships from the experiment in *a*, determined in the absence (○) or presence (●) of isoprenaline, or in the presence of both isoprenaline and DNDS (▲). Total cell capacitance = 123 pF; initial pipette resistance = 3.3 MΩ. In other cells, cAMP-activated current (compare with Fig. 3*A*) was reduced by 50–100% by 0.1 mM DNDS, and no further block was seen with 1 mM DNDS; 1 mM 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulphonate (SITS) had similar effects to 1 mM DNDS; neither DNDS nor SITS was effective on cells dialysed with 50 mM EGTA and 20 mM Cl^-.

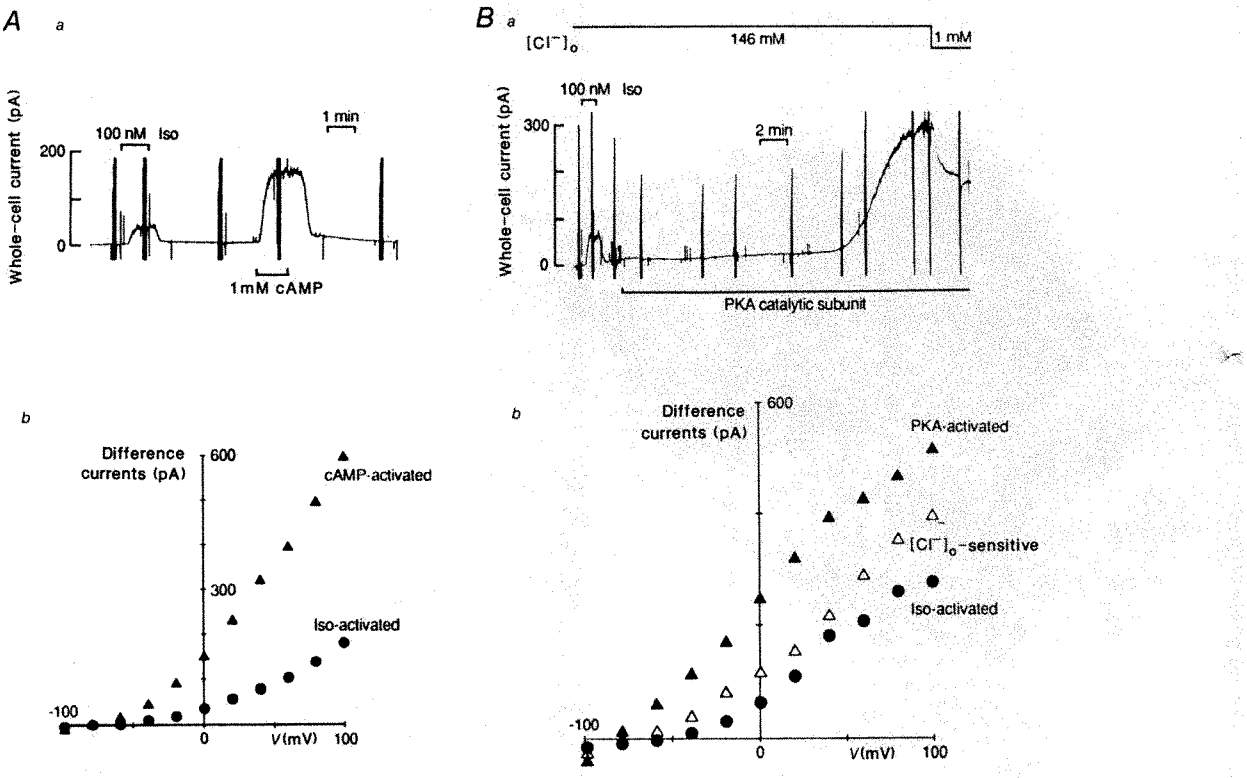

FIG. 3 Activation of Cl^- current by intracellular dialysis with either cAMP (*A*) or PKA catalytic subunit (*B*). *Aa*, Whole-cell current at 0 mV. Bars indicate exposure to isoprenaline or cell dialysis with 1 mM cAMP (in addition to 10 μM IBMX to diminish phosphodiesterase activity). The $[Cl^-]_{pip}$ was 0 mM. *b*, Steady-state *I–V* plots comparing isoprenaline-activated current (●) with cAMP-activated current (▲). Total cell capacitance = 150 pF; initial pipette resistance = 1.5 MΩ. *Ba*, Whole-cell current at 0 mV. The upper line marks a change of $[Cl^-]_o$ from 146 to 1 mM; the short horizontal bar shows the exposure to isoprenaline; the lower bar indicates the period of intracellular dialysis with a pipette solution containing 20 mM Cl^- (see Fig. 2*A*) and PKA catalytic subunit (0.3 mg ml^{-1}, specific activity 18 μmol min^{-1} mg^{-1}). Both pipette solutions included 2 mg ml^{-1} bovine serum albumin (BSA; Sigma, Fraction V). In the absence of PKA catalytic subunit, BSA had no effect on whole-cell current. The catalytic subunit of PKA was prepared from bovine heart as previously described[28]. *b*, Steady-state *I–V* plots comparing current activated by isoprenaline (●) or by PKA catalytic subunit (▲) with the $[Cl^-]_o$-sensitive component of PKA-activated current (△). Total cell capacitance = 98 pF; initial pipette resistance = 2.0 MΩ.

The lack of any Cl^--sensitive component of the steady membrane current without kinase activation (Figs 1 and 2) helps to explain the previous uncertainty about the existence of a Cl^- current in cardiac cells[9,19–21]. But the absence of earlier reports of the large Cl^- currents activated by β-catecholamine as described here is more puzzling, given recent studies of regulation of other currents in cardiac cells through the β-adrenoceptor/cAMP pathway[1,2,5,6,22,23]. Most of these studies focused on relatively large voltage- and time-dependent K^+ or Ca^{2+} currents, and so activation of the practically time-independent Cl^- current (Fig. 1*B*) was presumably either obscured by oppositely directed changes in a steady component of the Ca^{2+} channel current, or possibly ignored. Egan *et al.*[22,23] did report isoprenaline-induced inward shifts of current at large negative potentials, and outward shifts at large positive potentials, not unlike those described here, but they tentatively attributed them to enhanced Na^+ and K^+ currents, respectively, in part because the inward shift was diminished after replacing extracellular Na^+ by tetramethylammonium ions. Curiously, we found that replacement of all external Na^+ by 145 mM N-methyl-D-glucamine$^+$, but not by Cs^+, nearly abolished isoprenaline-activated inward and outward current.

Nevertheless, we believe that the currents reported here reflect Cl^--channel activity rather than electrogenic co-transport, because the currents could be large (up to 15 $\mu A\,cm^{-2}$ at +100 mV, assuming specific membrane capacitance = 1 $\mu F\,cm^{-2}$), they reversed near E_{Cl} at high $[Cl^-]_o$ and $[Cl^-]_i$ (Fig. 1*C*), and the holding-current shift was invariably accompanied by large-amplitude, low-frequency current noise (Figs 1–3). Analysis of this current noise and identification of corresponding unitary Cl^--channel currents in myocytes should settle this question.

The relationship between these putative cardiac Cl^- channels and those from the membranes of airway epithelial cells, known to be activated by PKA-mediated phosphorylation, remains unclear. The macroscopic cardiac Cl^- currents were (1) absent unless the cAMP pathway was activated, (2) showed almost no rectification with roughly symmetrical chloride concentrations, (3) showed little evidence of voltage-dependent gating, and (4) were irreversibly inhibited by DNDS. In contrast, epithelial Cl^- channels, whose regulation through PKA is defective in cystic fibrosis[12–15], are blocked reversibly by DNDS[8], have single-channel currents that show pronounced outward rectification with symmetrical chloride concentrations (but not after reconstitution in lipid bilayers[24]), and have an opening probability that is strongly influenced by voltage—they can be opened by large positive potentials in the absence of phosphorylation[14,15].

During β-adrenoceptor activation in myocytes, a large, maintained outward Cl^- current flows almost instantaneously (Fig. 1*B*) on depolarization (delayed K^+ current, in contrast, arises over hundreds of milliseconds[1,2,5,6]). This Cl^- current must therefore be expected to counter the depolarizing influence of the simultaneously enhanced inward Ca^{2+} current[1–4]. The Cl^- current should thus speed repolarization of the action potential and so might protect the heart from arrhythmogenic effects of strong sympathetic stimulation. □

Note added in proof. Since submission of this manuscript, R. D. Harvey and J. R. Hume[29] have reported a background current, with a reversal potential sensitive to $[Cl^-]_i$, which was elicited in guinea-pig ventricular myocytes by isoprenaline or by forskolin, and was therefore inferred to be regulated by cAMP.

Received 5 May; accepted 20 July 1989.

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ACKNOWLEDGEMENTS. This work was supported by the NIH (D.C.G., P.G.), the Irma T. Hirschl Trust (D.C.G.), and the Cystic Fibrosis Foundation (A.C.N.). We thank Peter Hoff for technical assistance.

Calcitonin gene-related peptide regulates calcium current in heart muscle

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THE influx of Ca^{2+} due to the transmembrane calcium current, I_{Ca}, has a fundamental role in cardiac pacemaker activity, in the action potential plateau and in excitation–contraction coupling. Both sympathetic and parasympathetic neurotransmitters can modulate I_{Ca} (ref. 1). Recent studies indicate that in both the cardiovascular[2–7] and the central nervous systems[8,9], nerve varicosities exist that contain a novel non-adrenergic, non-cholinergic peptide—calcitonin gene-related peptide (CGRP)[10]. Although CGRP is known to exert strong positive inotropic[3–5,7,11] and chronotropic[3,11] effects, as well as to cause vasodilatation[2,6,12,13], very little is known about the ionic mechanisms of these effects[14,15]. Here we report that CGRP dramatically increases I_{Ca} in single heart cells. Although this CGRP-induced increase in I_{Ca} resembles the effect of β-adrenergic agonists, our results demonstrate some significant differences between the effects of CGRP and these agonists: (1) the increase due to CGRP cannot be blocked by β-adrenergic antagonists; (2) the CGRP-induced effect is transient; and, (3) CGRP can inhibit isoproterenol-stimulated I_{Ca}. Our results provide the first electrophysiological evidence that CGRP can significantly modulate I_{Ca} in the heart, and suggest a new additional mechanism for the neurogenic control of cardiac function.

Our initial experiments consistently showed that, in single atrial myocytes from bullfrog hearts, CGRP increased I_{Ca} in a potent (threshold CGRP concentration, 10^{-9} M), dose-dependent and reversible fashion. In CGRP of concentration 3×10^{-7} M, I_{Ca} recorded at +10 mV increased from 108 ± 27 pA to 791 ± 124 pA (mean ± s.e.m., $n = 12$, Fig. 1). Although this ~8-fold increase in I_{Ca} resembled the response of these cells to isoprenaline[16], the CGRP-induced effect could not be blocked by the β-adrenergic antagonist, propranolol (3×10^{-7} M), indicating that it was not mediated by stimulation of β-adrenoceptors. This is consistent with the recent finding that specific CGRP binding-sites are present in several tissues, including those of the heart[17–19].

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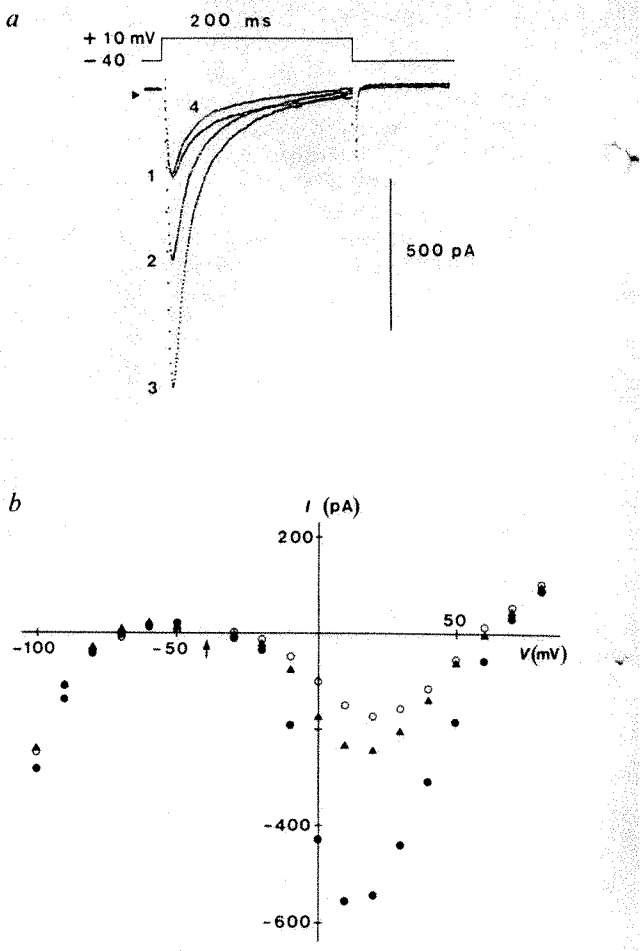


FIG. 1 Demonstration of the CGRP-induced increase in I_{Ca} in frog atrial myocytes. *a*, Superimposed tracings of membrane current in control Ringer's solution (1) and CGRP-containing Ringer's solutions (2, 3). CGRP concentrations were 10^{-8} M (2) and 3×10^{-7} M (3). I_{Ca} records were obtained from a holding potential (V_H) of −40 mV by applying 200-ms depolarizing test pulses to +10 mV at 0.2 Hz. Propranolol (3×10^{-7} M) was present to block β-adrenergic effects. Note that I_{Ca} increased after the application of CGRP, and that these effects were reversible following washout of the peptide (4). The addition of $CdCl_2$ (3×10^{-4} M) blocked these effects completely. The zero current level is indicated by a triangle. *b*, *I–V* relation for I_{Ca} and the inwardly rectifying potassium current (I_{K1}). V_H was −40 mV (shown by an arrow) and 200-ms test pulses were applied in 10-mV increments at 0.2 Hz. The amplitude of I_{K1}, which was measured at the end of 200-ms test pulse, was not significantly changed by CGRP. Membrane currents were recorded from the same cell in control solutions (○) and solutions containing CGRP at concentrations of 10^{-8} M (▲) and 3×10^{-7} M (●).

METHODS. Atrial myocytes were isolated from bullfrog *Rana catesbeiana*, or from rabbit hearts using methods described previously[20]. In brief, frog atrium was dissected out and cut into pieces (1 mm × 1 mm) in low Ca^{2+}-modified frog Ringer's solution (8.0 μM $CaCl_2$), and incubated in this solution containing collagenase (240 units ml^{-1}, Yakult, Japan) and trypsin (3,600 units ml^{-1}, Type III, Sigma) for 45 min at room temperature. After being rinsed with 0.1% albumin, the tissue was further treated with collagenase (120 units ml^{-1}) for 40–60 min. This procedure gave a satisfactory number of viable single cells. Rabbit atrial myocytes were obtained by perfusing a high K–low Cl solution (KB solution) containing collagenase (58–77 units ml^{-1}) through the coronary circulation for 20 min at 37 °C (Langendorff perfusion). Composition of KB solution (mM): Glutamic acid mono-K salt (50.0), KCl (25.0), taurine (10.0), KH_2PO_4 (10.0), EGTA (0.5), glucose (10.0), HEPES (10.0), $MgCl_2$ (3.0), pH 7.4, adjusted with KOH. Electrode solution for frog (mM): K-aspartate (90.0), KCl (20.0), EGTA (5.0), HEPES (5.0), ATP-2Na (3.0), $MgCl_2$ (2.0), pH 7.2, adjusted with KOH; for rabbit (mM): K-aspartate (120.0), KCl (30.0), EGTA (1.0), HEPES (5.0), ATP-2Na (4.0), $CaCl_2$ (0.486), $MgCl_2$ (1.0), pCa 7.0, pH 7.2, adjusted with KOH. In all experiments membrane current was recorded from single isolated myocytes by a single electrode patch-clamp technique in the whole-cell configuration[20,26]. Membrane current was digitized with a sampling speed of 2.5 KHz. Composition of the modified Ringer's solution for frog (mM): NaCl (110.0), KCl (2.5), glucose (10.0), HEPES (5.0), $CaCl_2$ (2.5), $MgCl_2$ (5.0), pH 7.4 adjusted with NaOH. Composition of the Tyrode's solution for rabbit (mM): NaCl (135.0), KCl (5.4), glucose (5.5), HEPES (5.0), $CaCl_2$ (1.8), $MgCl_2$ (0.5), NaH_2PO_4 (0.33), pH 7.4 adjusted with NaOH. Drugs were dissolved in these solutions and applied by superfusion at ~1.5 ml min^{-1}. The volume of the recording chamber was ~0.5 ml. Measurement of the *I–V* relation was started 2.5 min after application of each dose of the peptide when I_{Ca} had reached a steady level. The data were obtained in 1.5 min. During this period, there was no measurable decline in the current at either high or low doses of CGRP. Drugs used in this study were rat CGRP (Peninsula Laboratories, California), (-)isoprenaline (Sigma Chemical, Missouri), DL-propranolol (Sigma), cAMP (Sigma), forskolin (Sigma) and cadmium chloride (Sigma).

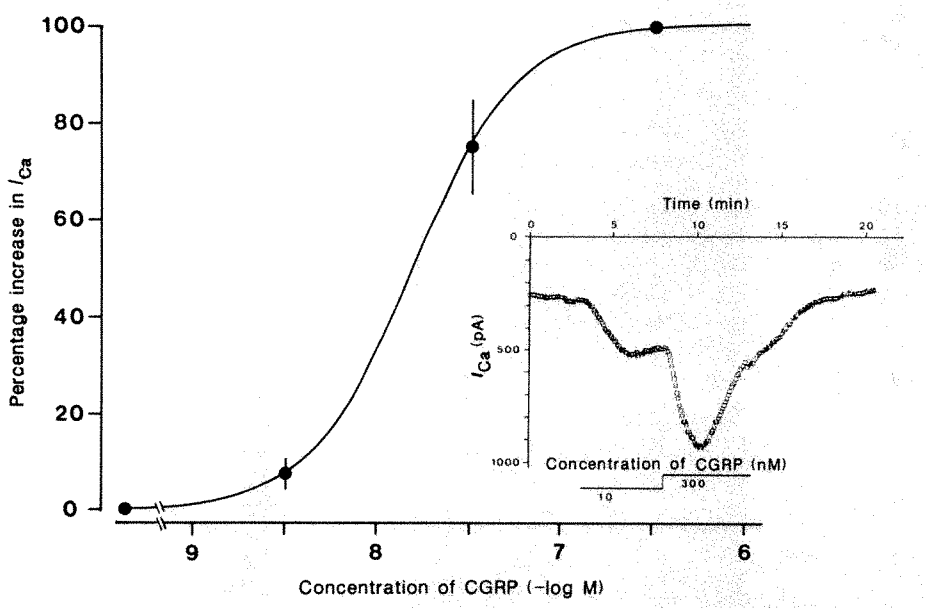


FIG. 2 Dose–response curve for the CGRP-induced enhancement of I_{Ca} in bullfrog atrial myocytes. In each cell, three different concentrations of CGRP were applied cumulatively by rapidly increasing CGRP concentration just after I_{Ca} had reached a steady level for each dose. The increase of I_{Ca}, measured at its maximum level for a particular dose, was expressed as a percentage of the maximum increase (~8-fold at a CGRP concentration of 3×10^{-7} M) in each cell. The entire experiment was completed within 6 min. Since the effects of CGRP on I_{Ca} did not decline significantly during this short period (except for the maximal dose), these results provided an indication of the steady-state dose–response relationship. Test pulses of 200 ms to +10 mV from the V_H of −40 mV were applied at 0.2 Hz. Each point represents the mean ± s.e.m. of four different experiments. The solid line shows the best fit Langmuir relationship; the ED_{50} was $1.64\pm0.55\times10^{-8}$ M ($n=4$). Inset, comparison of the time course of the stimulation of I_{Ca} by low (10^{-8} M) and high (3×10^{-7} M) doses of CGRP. When relatively high ($>10^{-7}$ M) doses were applied for a long period (5 min), the response was found to be transient; I_{Ca} declines quickly after reaching its maximum.

The current-voltage (I-V) relation for I_{Ca} in Fig. 1*b* illustrates the effects of CGRP at concentrations of 10^{-8} M and 3×10^{-7} M. The increase in I_{Ca} was dose-dependent without a significant change in its voltage dependence. Moreover, under these conditions, the main effect of CGRP seemed to be on I_{Ca}, because (1) the inwardly rectifying background current, I_{K1}, was not changed significantly, as indicated by the lack of effect at potentials negative to −50 mV, (2) the CGRP effect was completely blocked by $CdCl_2$ (3×10^{-4} M), and (3) the delayed rectifier K^+-current, I_K, is activated very slowly in this preparation[20] and, therefore, is very small when short (200 ms) depolarizations are applied. Under different conditions, I_K, which is responsible for initiation of repolarization in these cells[20], can also be increased by CGRP. This effect can be demonstrated in experiments in which much longer depolarizations are used[20] so that I_K is activated more strongly (data not shown).

We obtained more information about the physiological importance of the CGRP-induced increase in I_{Ca} by examining its dose-dependence and time course in detail. Figure 2 shows the dose-response relation for the observed effect of CGRP. The threshold CGRP concentration was $\sim10^{-9}$ M, and the 50% effective dose (ED_{50}) was $1.64\pm0.55\times10^{-8}$ M (mean ± s.e.m., $n=4$). The maximum response (~8-fold increase) was observed at 3×10^{-7} M ($n=4$). It is interesting that the dose-dependence of this effect is similar to the dose-dependence of the positive inotropic ($ED_{50}=0.6$–5.0×10^{-8} M)[3–5,11,21] and chronotropic ($ED_{50}=0.7$–5.0×10^{-8} M)[3,11] effects of this peptide. A similar CGRP-induced increase in I_{Ca} has been recorded in cells from rabbit atrium. In two experiments, CGRP at a concentration of 3×10^{-7} M increased I_{Ca} at 0 mV by 1.7- and 1.4-fold, respectively (data not shown).

Measurement of the dose-dependent effects of CGRP on I_{Ca} showed an important difference between this response and the one due to isoprenaline. Although the stimulatory effect of CGRP on I_{Ca} was well-maintained at CGRP concentrations of 3×10^{-8} M or lower, it was transient at concentrations greater than $\sim10^{-7}$ M (Fig. 2, inset; Fig. 3). In contrast, even maximal effects of isoprenaline were well-maintained (Fig. 3). At a CGRP concentration of 3×10^{-7} M, I_{Ca} reached a maximum after ~160 s (156 ± 14 s, mean ± s.e.m., $n=6$) and then declined to ~50% (45.6 ± 5.8%, $n=4$) of this value within 3 min (Fig. 3). This decrease was not due to run-down of I_{Ca} because, after the CGRP effect had declined to a steady level, application of isoprenaline (10^{-6} M) increased I_{Ca} substantially (Fig. 3).

Further experiments attempted to obtain information about the intracellular mediator(s) of the CGRP response. CGRP stimulates adenylyl cyclase[5,18,21] in the heart. The enhancement of I_{Ca} may, therefore, be mediated by increases in intracellular cyclic adenosine-3′,5′-monophosphate (cAMP), even though CGRP cannot activate sarcolemmal β-adrenergic receptors. This was tested by either (1) increasing intracellular cAMP by dialysis from the recording pipette, or (2) pre-treating the myocytes with forskolin, a direct activator of adenylyl cyclase. When the cells were perfused intracellularly with cAMP (10^{-4} M), I_{Ca} was enhanced maximally[16] (1,415 ± 418 pA, mean ± s.e.m., $n=3$). Subsequent application of CGRP (3×10^{-7} M) did not increase I_{Ca} any further (1,460 ± 411 pA, $n=3$, Fig. 4*a*). Forskolin (5×10^{-7} M) alone did not affect I_{Ca}; it significantly potentiated the stimulatory effect of CGRP, however. For example, CGRP (10^{-8} M) alone gave an increase in I_{Ca} from 78 pA to 216 pA; in the same cell after pre-treatment with forskolin, CGRP (10^{-8} M) increased I_{Ca} to 417 pA (Fig. 4*b*). These results suggest that the enhancement of I_{Ca} by CGRP is mediated by cAMP, following stimulation of adenylyl cyclase.

One possible explanation for the relatively rapid decline in I_{Ca} after CGRP-induced enhancement is that the CGRP receptors desensitize quickly[22]. Consistent with this is the finding that, when brief applications of low doses of CGRP ($\leq3\times10^{-8}$ M) were repeated at intervals of >20 min, the stimulatory effect was reproducible. Higher doses of CGRP ($>10^{-7}$ M), however, caused significant desensitization. For example, when I_{Ca} had declined in the presence of CGRP (3×10^{-7} M), and the peptide was then washed out and re-applied after 20 or 30 min, the increase in I_{Ca} was only 30% of that of the first response (data not shown). Moreover, CGRP partially inhibited isoprenaline stimulation of I_{Ca} (Fig. 4*c*). This inhibitory effect was statistically significant ($P<0.001$). Further investigation is needed before the intracellular mechanisms for the CGRP-induced transient stimulation of I_{Ca} can be understood. This stimulation helps to explain previously reported transient effects of CGRP, such as its enhancement of contraction and its stimulation of transmitter release[11,23].

Our results provide the first direct evidence that CGRP has a significant stimulatory effect on I_{Ca} in the heart. We show that this effect is reversible, dose-dependent, and resistant to blockade by β-adrenergic antagonists. The CGRP-induced increase in I_{Ca} seems to be mediated by stimulation of adenylyl cyclase that is coupled to a specific CGRP receptor. The increase in I_{Ca} can explain the positive inotropic and chronotropic effects of this peptide, because transmembrane calcium influx is essential for both contraction and the pacemaker activity[24]. Combined with previous evidence of CGRP-containing nerve varicosities in the atrial muscle[3–5,7], and release of this peptide during normal[3,4,7] and ischaemic[25] conditions, the electrophysiological

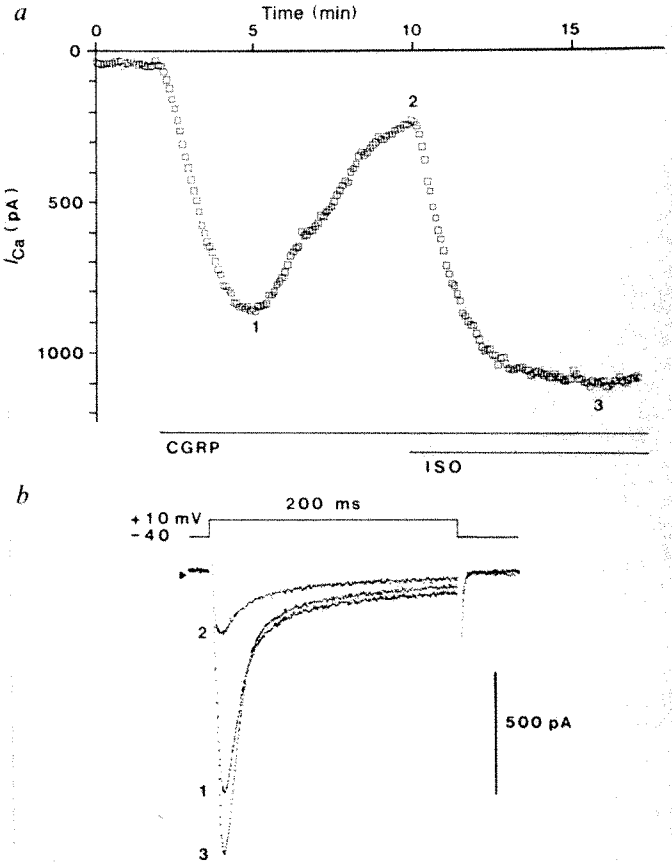
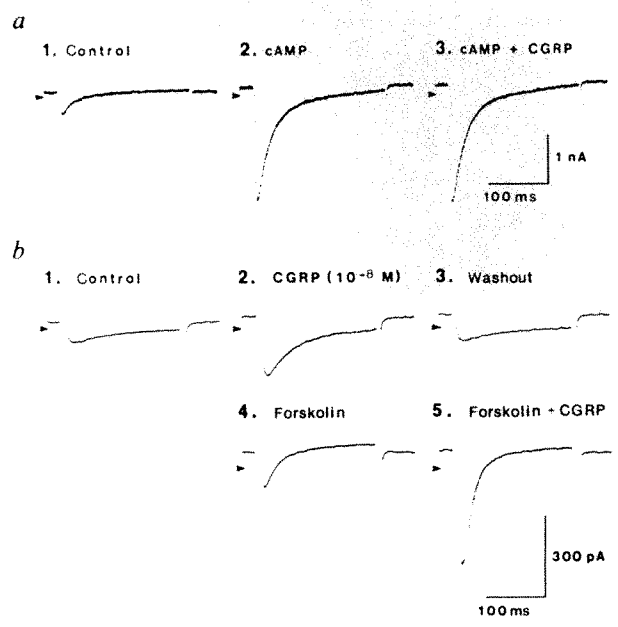


FIG. 3 Comparison of the time-courses of the effects on I_{Ca} of maximal doses of CGRP (3×10^{-7} M) and isoprenaline (ISO) (10^{-6} M). *a*, Time courses of the change in the peak size of I_{Ca}. The horizontal bars show the period during which each drug was applied. Note that the CGRP effect is transient whereas that of isoprenaline is maintained. *b*, Superimposed records, taken at the time indicated in *a*. The zero current level is indicated by a triangle. CGRP increased I_{Ca} (from 125 ± 35 pA to 754 ± 121 pA, mean ± s.e.m., $n=8$) (1), but this peak response rapidly decayed (2). After the CGRP-induced decline of I_{Ca} had reached a steady level, isoprenaline was added in the presence of CGRP. As shown in *a*, isoprenaline still gave a significant increase in I_{Ca}; I_{Ca} exceeded that recorded previously in the presence of CGRP (3). The same result was obtained when isoprenaline was applied in the absence of CGRP, after the decline of I_{Ca}.



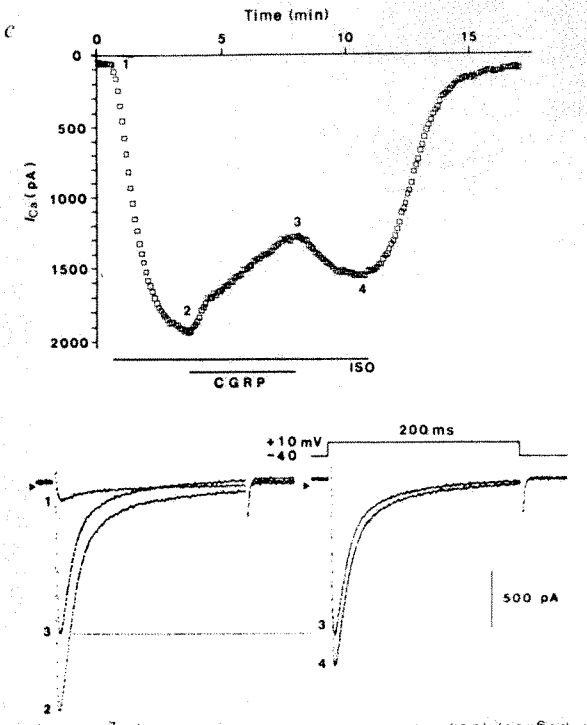


FIG. 4 *a*, Tests for involvement of intracellular cAMP in the CGRP-induced increase in I_{Ca}. Cyclic AMP (10^{-4} M) was included in the pipette before the whole-cell impalement was made, and the control record (1) was taken immediately after the impalement. Cyclic AMP maximally increased I_{Ca} (2) and the subsequent application of CGRP (3×10^{-7} M) did not increase I_{Ca} any further (3). *b*, Potentiation of the effect of CGRP by forskolin (5×10^{-7} M). A low dose of CGRP (10^{-8} M) was applied repeatedly at intervals of 30 min. Forskolin was applied 5 min before the second application of CGRP. Forskolin alone did not increase I_{Ca} (4), but it strongly potentiated the effect of the subsequent application of CGRP (2, 5). I_{Ca} records in the presence of CGRP were taken after it had increased maximally at each peptide application. Triangles denote the zero current level in all panels. *c*, Inhibitory effect of CGRP (3×10^{-7} M) on I_{Ca} stimulated by isoprenaline (ISO) (10^{-6} M). Upper panel shows the time course of the changes in I_{Ca}. Lower panel shows raw data recorded at the times marked by the numbers in the upper panel. Isoprenaline (10^{-6} M) increased I_{Ca} maximally within 2 min (2). This effect was persistent; there was no detectable decay 3 min after the maximal level was reached (98.0 ± 3.2%, mean ± s.e.m., $n=4$, Fig. 3*a*). In contrast, I_{Ca} declined quickly when CGRP (3×10^{-7} M) was added just after it had reached its maximum size in the presence of isoprenaline (10^{-6} M) (3); 3 min after the application of CGRP, I_{Ca} had decreased to 72.1 ± 3.4% of the initial maximum size ($n=5$, $P<0.001$, compared with the value of I_{Ca} in the absence of CGRP). This inhibitory effect was partly reversed when CGRP was washed out (4).

actions of CGRP that we have identified provide important new insights into neurogenic control of the heart in both normal[3,4,7] and pathological[25] conditions. □

Received 26 January; accepted 10 July 1989.

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ACKNOWLEDGEMENTS. We thank the Medical Research Council of Canada, the Heart and Stroke Foundation of Alberta, and the Alberta Heritage Foundation for Medical Research for financial assistance.

Altering DNA-binding specificity of GAL4 requires sequences adjacent to the zinc finger

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MANY eukaryotic proteins involved in transcriptional regulation contain within their DNA-binding domains a polypeptide loop (the zinc finger) which interacts with DNA[1,2]. In proteins possessing multiple zinc fingers, including TFIIIA[3], Sp1[4,5], SWI5[6] and oestrogen/glucocorticoid receptors[7], the region containing the zinc fingers confers DNA-binding specificity. By contrast, our results demonstrate that all but one of the 28 amino acids encompassing the single zinc-finger region of GAL4, the yeast transcriptional activator, can be replaced with the analogous zinc-finger region from another yeast-activator protein, PPR1, without changing the DNA-binding specificity of GAL4. A 14-amino-acid region adjacent to the zinc finger is necessary for determining specific recognition of DNA sequences.

To determine the role of the GAL4 zinc finger in specific DNA binding, increasing segments of the 28-amino-acid cysteine-rich zinc-finger region of GAL4 protein have been

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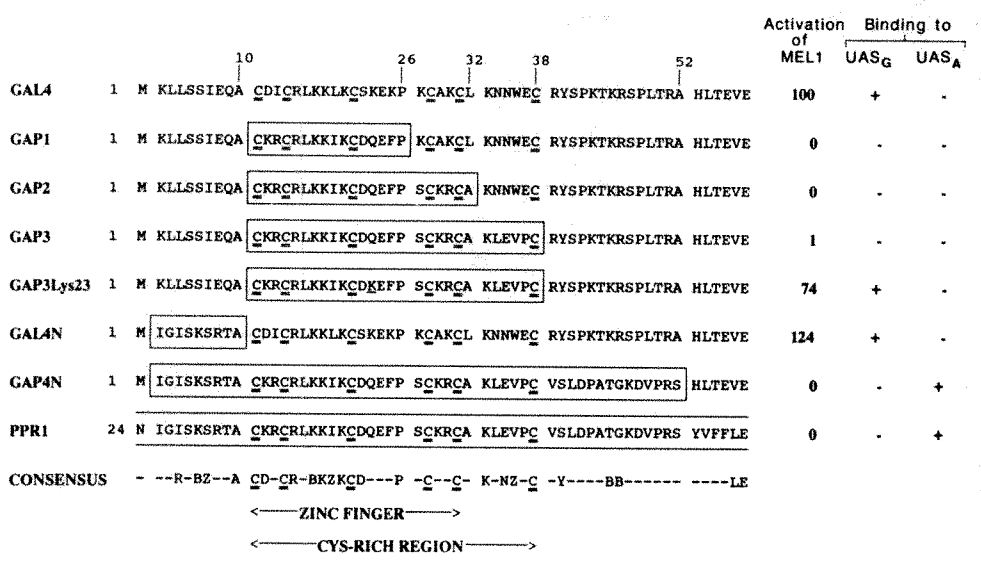

FIG. 1 *MEL1* activation and DNA-binding activities of the GAL4–PPR1 hybrid proteins. PPR1 sequences are boxed. The six conserved cysteines are double underlined. Two pairs of cysteines (Cys_1 and Cys_2, Cys_4 and Cys_5) are thought to coordinate an atom of zinc to form the zinc finger. Numbers on the side refer to the position in the protein of the first amino acid in the line. Numbers on top refer to the position in GAL4 of the last PPR1 amino acid in the hybrid protein. Lys23 in GAP3Lys23 is underlined. The consensus sequence includes amino acids which are conserved in at least half of the 10 proteins from the C_6 class including GAL4[13], LAC9[23], qa-1F, QUTA[24], PPR1[9], ARGRII, HAP1, LEU3, MAL6 and PDR1[25]. Standard one letter codes are used, plus Z for the hydrophobic amino acids W, I, V and L, and B for basic amino acids R or K. *MEL1* (α-galactosidase) activities of the hybrids are shown as a percentrage of the activation by wild-type GAL4. The DNA-binding behaviour is shown as either + or − determined by electrophoretic mobility shift assays (Fig. 2).

METHODS. The starting plasmid, *GAL4*/pUC119, consisted of a *Bam*HI/*Hin*dIII fragment containing the wild-type *GAL4* gene cloned into pUC119[26]. Single-stranded DNAs were prepared as described[26] and used as templates for site-directed mutagenesis using an Amersham *in vitro* mutagenesis kit. Primers used to direct mutagenesis were synthesized on an Applied Biosystems Model 380B DNA synthesizer. The sequences of the primers are available upon request. The *Bam*HI–*Xho*I fragments from the hybrids were cloned into the 2 μ plasmid *GAL4*/YEp351 containing the selectable marker *LEU2* gene, replacing the wild-type *Bam*HI-*Xho*I fragment. The nine amino acids *N*-terminal to the first cysteine of the zinc finger of *GAL4* were converted to the analogous *PPR1* sequence. The *GAL4* DNA sequences of the GAP4 hybrid encoding into the first nine amino acids on a *Bam*HI–*Sph*I fragment were replaced with the PPR1 sequences on a *Bam*HI–*Sph*I fragment. All sequence changes by mutagenesis were confirmed by sequencing[27]. The *PPR1* gene contained on a *Pvu*II–*Pvu*II fragment from the plasmid *PPR1*/pUC8 (provided by R. Losson) was cloned into the *Sma*I site of YEp351. A *Bam*HI–*Hin*dIII fragment containing *GAP3* was cloned into vector YCp50f1. This plasmid contains the f1 ori region from plasmid pVT100-L[28] in YCp50. The yeast strain SC30 (*a gal4*$^{\Delta}$ *ura3-52 leu2-3 112*) containing *GAP3*/YCp50f1 was plated on to minimal media lacking uracil containing 2% galactose. Plasmids from revertants were isolated from yeast[29], transformed into *E. coli*, checked by restriction enzyme digests, and sequenced[27,30]. Putative revertant plasmids were transformed into YJ0 (*a gal4*$^{\Delta}$ *ura*3-52 *leu*2-3 112 *his*3$^{\Delta}$ *ade1 gal80*$^{\Delta}$ *MEL1*). The *Bam*HI–*Xho*I fragment of the revertant was cloned into plasmid *GAL4*/351. The α-galactosidase (*MEL1*) assay was essentially as reported[10]. The YJ0 strain carrying the *GAL4*, *GAP* or *PPR1* plasmids was grown in minimal media lacking leucine. The carbon source was 3% glycerol and 2% lactic acid. All plasmids were tested from level of production of GAP proteins in a B80^{s} strain (*α GAL4 GAL80*^{s}*-100 ura3-52 leu2-3 112 MEL1*) by assaying growth rate on galactose[17]. *PPR1* or YEp351 plasmids did not allow the strain to grow on galactose.

replaced, by *in vitro* mutagenesis, with the analogous sequences of PPR1, an activator of two genes involved in pyrimidine biosynthesis, *URA1* and *URA3* (refs 8, 9). The plasmids encoding the hybrid proteins, designated GAP or GAL4N (Fig. 1) were tested in a *gal4*$^{\Delta}$ strain for their ability to activate expression of the *MEL1* (α-galactosidase) gene which is normally under the control of GAL4. Neither GAP1 nor GAP2 activated *MEL1* expression. The yeast strain containing the plasmid encoding GAP3, in which all of the cysteine-rich region of GAL4 was replaced, possessed 1% of the *MEL1* activity of the strain containing the GAL4-encoded plasmid. A *gal4*$^{\Delta}$ strain has less than 0.1% background activity[10].

Hybrid protein levels were determined by assaying another function of GAL4, namely its ability to interact through the C terminus[11,12] with the GAL80^{s} negative regulatory protein (Fig. 1 legend). All of the hybrid proteins used in this study were stably produced at high enough levels to titrate out the repressive effects of GAL80^{s} protein, reflecting at least 50% wild-type protein levels. The low or negligible activity of GAP1, GAP2 and GAP3 is, therefore, most probably due to a disrupted DNA-binding structure and not low protein stability or production.

A mutation in the *GAP3* gene was found which conditioned higher levels of *GAL* and *MEL1* gene expression. Mutants of a *gal4*$^{\Delta}$ strain containing GAP3 were selected on plates containing galactose as the sole carbon source. A *GAP3* mutant which grew well on galactose was found to be a C to A transversion at nucleotide 509 (by the numbering of Laughon and Gesteland[13]) resulting in a predicted change of Gln 23 to Lys near the tip of the zinc finger. Lysine is the amino acid found at this position in GAL4. On a multicopy plasmid, GAP3Lys23 activates 74% of the level of *MEL1* gene expression that is activated by GAL4.

The DNA binding properties of the hybrid proteins were studied directly by electrophoretic mobility shift assays[14,15] using either a GAL4 DNA-binding site, UAS_G, or a PPR1 DNA-binding site, UAS_U. This *in vitro* assessment of the hybrid proteins was important, because transcriptional activation and DNA binding are separable functions[16]. Extracts containing GAP1, GAP2 or GAP3 proteins did not form detectable complexes with either UAS_G or UAS_U (data not shown). However, specific binding by GAP3Lys23 to UAS_G was detected (Fig. 2*a*, lanes 4 and 5). The putative GAP3Lys23–UAS_G complex reacted with antiserum against GAL4 protein[17] (Fig. 2*a*, lane 6), but not with preimmune serum (Fig. 2*a*, lane 5), confirming that the complex contained the GAL4 hybrid protein. Thus, the GAL4 protein containing most of the PPR1 zinc-finger region retained GAL4 DNA-binding specificity and ability to activate *MEL*1 to high levels.

The contribution to DNA binding specificity by amino acids directly preceding the zinc finger was determined by replacing the 9 N-terminal amino acids of GAL4 with the analogous PPR1 sequences. The hybrid GAL4N activated the *MEL*1 gene to a level 124% that of wild-type GAL4 and retained the DNA binding specificity of GAL4 (Fig. 2*b*, lane 3) demonstrating that these 9 amino acids are not involved in DNA-binding specificity.

To locate the sequences required for specific recognition of DNA, we converted an additional 14-amino-acid region immediately following the sixth cysteine of the zinc-finger region to give the hybrid GAP4N. These 14 amino acids of PPR1 switched the DNA-binding specificity of GAL4 to that of PPR1 as GAP4N bound specifically to UAS_U (Fig. 2*c*, lane 3) but did not bind

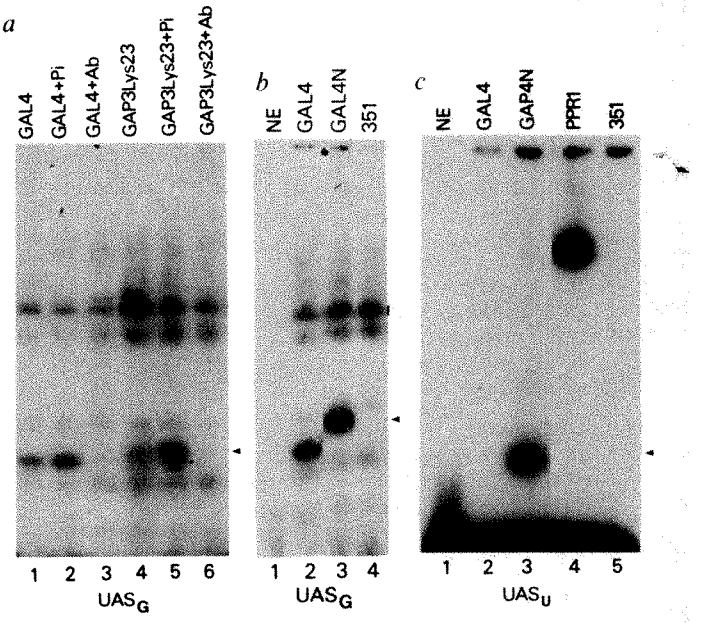

FIG. 2 DNA-binding specificity of GAL4–PPR1 hybrid proteins. *a*, Binding of GAP3Lys23 to UAS_G and specific titration of the GAP3Lys23–UAS_G complex with anti-GAL4 antiserum. UAS_G was preincubated for 30 min with extracts containing GAL4 (lanes 1–3), or GAP3Lys23 (lanes 4–6). Then 1 μl of preimmune serum (Pi, lanes 2 and 5) or 1 μl of immune serum (Ab, lanes 3 and 6) was added to the tubes. After an additional 30 min incubation, the protein–DNA complexes were resolved on an agarose gel. The arrowhead points to the GAL4- and GAP3Lys23-specific complexes. *b*, Binding of GAL4N to UAS_G was incubated with buffer alone (lane 1, NE) or with extracts containing GAL4 (lane 2), GAL4N (lane 3) or YEp351 (lane 4). The arrowhead points to the GAL4N–UAS_G complex. *c*, Binding of GAP4N to UAS_U. UAS_U was incubated with buffer alone (lane 1, NE) or with extracts containing GAL4 (lane 2), GAP4N (lane 3), PPR1 (lane 4) or YEp351 (lane 5). The arrowhead points to the GAP4N–UAS_U complex.

METHODS. Yeast extracts were prepared using a modification of the procedure of Bram and Kornberg[31]. All steps were performed at 0–4 °C and buffer A(50) did not include pepstatin A or leupeptin. Typically, a 40 ml culture of yeast cells was grown at 30 °C to $3–5 \times 10^7$ cells ml^{-1}. The cells were collected by centrifugation (10 min, 3,000*g*) and resuspended in buffer A(50) plus 0.48 M $(NH_4)_2SO_4$. The cells were lysed by adding acid-washed glass beads (0.45 mm) and vortexing for 4×30 s at maximum speed. The resulting crude extract was centrifuged at 5,000*g* for 10 min and the supernatant collected. Solid ammonium sulphate (0.46 g ml^{-1}) was immediately added to the supernatant. The solution was gently mixed until the ammonium sulphate was completely dissolved. The precipitated protein was collected by centrifugation at 10,000*g* for 15 min and resuspended in 200 μl buffer A(50). The resuspended protein was dialysed against 500 volumes of buffer A(50) for at least 2 h. The resulting extracts were stored at −20 °C and contained 8–10 mg protein per ml. Synthetic oligonucleotides containing the sequence 5′-AATTCGGGTGACAGCCCTCCGAAGGGTAC-3′ and 5′-CCTTCGGAGGGCTGTCACCCG-3′ (UAS_G) or 5′-AATTCATTCGGTAATCTCC-GAACGGTAC-3′ and 5′-CGTTCGGAGATTACCGAATG-3′ (UAS_U) were annealed and labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Each binding reaction contained buffer A(50) without protease inhibitors, 0.75 μg of sonicated salmon sperm DNA, 0.8 ng [^{32}P]DNA, and 5–15 μl yeast extract in a total volume of 20 μl. The reactions were incubated on ice for 45 min followed by addition of 1 μl sample dye. The reactions were immediately loaded on to a 2.5% NuSieve agarose (FMC Bioproducts) + 0.5% low melting temperature agarose (International Biotechnologies) gel in 0.5 × TBE (0.045 M Tris-borate, pH 8.0, 0.045 M boric acid, 0.001 M EDTA) and electrophoresed at 200 V at 3 °C. After electrophoresis the gel was fixed for 30 min in 10% trichloroacetic acid, blotted dry between paper towels overnight, and dried on a vacuum gel dryer with 45 °C heat. Autoradiography was performed with Kodak X-Omat film at −70 °C with an intensifying screen for 1–3 days.

the UAS_G (data not shown). The putative GAP4N–UAS_U complex reacted with GAL4 immune but not preimmune sera (data not shown) confirming that GAL4 protein sequence was in the complex. As expected, GAP4N failed to activate *MEL*1 expression, indicating that it did not bind to and activate at UAS_G, to even low levels. The binding activity of GAP4N demonstrates that sequences necessary for the specific recognition of UAS_U by PPR1 are encoded in the 14 amino acids flanking the sixth cysteine of the zinc-finger region.

The zinc-finger structure has been shown to confer the ability of some transcriptional activator proteins to interact specifically with DNA. We show that for two members of the C_6 class of zinc-finger proteins[2], most of the important determinants of specificity are not located within the zinc finger. The zinc fingers in at least some of these proteins may perform a common function such as non-sequence-specific interactions with the DNA. In this regard, a region of the adenovirus E1a protein[18] containing one zinc finger and the second zinc finger in the glucocorticoid/oestrogen receptors[7] have been implicated in non-sequence-specific interactions with DNA. Both the second finger from the steroid receptors and the C_6 class fingers contain several basic residues found primarily on one half of the finger, which may make contacts with the DNA phosphate-backbone.

We have shown that amino-acid sequences necessary for specific recognition of UAS_U are located within the 14 amino acids following the sixth cysteine. Mutations within this region of GAL4 abolish DNA binding[19,20]. Two differences distinguish these 14 amino acids from the three amino acids important in determining the specificity of the glucocorticoid/oestrogen receptors[21]. The three amino acids are located close to the second pair of cysteines of the first zinc finger, whereas the 14 amino acids of GAL4 are located a greater distance (seven amino acids) after the second pair of cysteines. Furthermore, most of the 14 amino-acid region of GAL4 has been predicted to form a random structure[22], whereas the three amino acids important in receptor binding are located within a proposed α-helix.

Homology within the 14 amino acids between two evolutionarily related proteins which recognize the same DNA sequence, *GAL4* and *LAC9* (the *GAL4* homologue from *Kluyveromyces lactis*) indicated the importance of these sequences in DNA binding specificity[23]. By contrast, there is little homology between GAL4 and other proteins in the C_6 class within this region, even though all C_6 class proteins have homology within the cysteine-rich region (Fig. 1, consensus). Interestingly, two other evolutionarily-related proteins qa-1F and QUTA isolated from two different fungi share a region of extensive homology in approximately the same position as the specificity region of GAL4 and LAC9 (ref. 24).

Our results suggest we can roughly partition the GAL4 DNA-binding region into two functional domains, supporting an earlier model of how C_6 proteins bind DNA[23]. It should be emphasized, however, that the DNA-binding region may have complex interactions between different parts of itself which preclude complete separation of a functional domain. In support of this, two mutations in *gal4*$^{\Delta}$—found 10 and 26 amino acids C-terminal to the zinc finger—showed growth correction on galactose in the presence of high concentrations of Zn(II). It was hypothesized that these alterations were manifestations of direct interaction between amino acids in the zinc finger and those downstream[20]. It is possible that Lys23 in the GAP3 mutant makes a structural contact with another part of the GAL4 protein or contributes to DNA-binding specificity. Further replacements of the DNA-binding domain of GAL4 are underway to locate the key amino acids determining binding specificity. □

Received 26 June; accepted 21 July 1989.

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ACKNOWLEDGMENTS. This work was supported by grants from Glaxo, Inc. and NIH to S.A.J. We thank Scott Langdon for development of the GAL4 electrophoretic mobility shift assay, Regine Losson for the *PPR1* plasmid and unpublished results, and John Salmeron, S. Langdon and Jackie Bromberg for suggestions.

Two tobacco DNA-binding proteins with homology to the nuclear factor CREB

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THE 35S promoter of the cauliflower mosaic virus (CaMV) contains a tandem repeat of the sequence TGACG in the region −83 to −63. This 21-base pair (bp) sequence, called *as-1*, is involved in root expression of the 35S promoter. When inserted in a promoter of a gene expressed specifically in photosynthetic tissues, *as-1* confers high level expression in roots[13]. We have described a factor, ASF-1, that binds specifically to *as-1 in vitro*. There is a good correlation between ASF-1 binding affinity to *as-1* related sequences *in vitro* and the function of these sequences *in vivo*. These results strongly suggest that ASF-1 is responsible for the function of *as-1* (ref. 13). Here we report the isolation of tobacco complementary DNA clones encoding two TGACG-sequence-specific binding-proteins (TGA1a and TGA1b). Sequence analysis of the cDNA clones shows that both proteins contain a basic region that shows high homology to a stretch of basic amino acids in the nuclear factors CREB, GCN4, and c-Jun[1–4] to a 'leucine-zipper' region[5]. On the basis of binding specificity we propose TGA1a to be a good candidate for ASF-1.

We have reported previously that tobacco nuclear extract contains a factor, ASF-1, that binds to the *as-1* sequence of the 35S promoter[13]. Mutations in the TGACG motifs of *as-1* leads to a drastic decrease in this binding activity. The results shown in Fig. 1*a* confirmed these findings and show that at the extract concentration used, two DNA-protein complexes were obtained with *as-1*. Only the faster-migrating complex (marked by an arrow head), however, is seen at a lower extract concentration[13]. This faster-migrating complex was also formed with two other TGACG-containing sequences, *hex1* and *nos1*, present in the upstream region of the wheat histone H3 gene and the promoter of the gene encoding nopaline synthase, respectively[6,7] (Fig. 1*a*). In both cases, mutations in the TGACG motifs reduced ASF-1 binding. An additional DNA-protein complex (marked by an open circle) obtained with the *hex1* probe is competed by only *hex1*, but not by *as-1* or *nos1* (not shown).

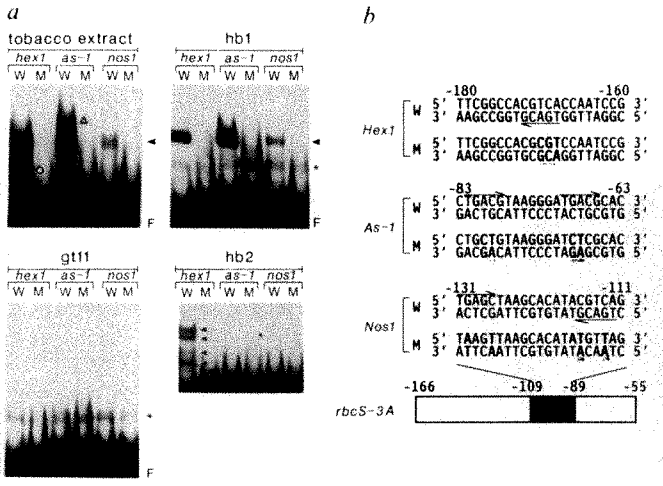

FIG. 1 DNA binding specificity of ASF-1, TGA1a, and TGA1b. *a*, Gel retardation assays[8] with tobacco nuclear extract and lysogen extracts from hb1, hb2, and λgt11. For hb2, the lower part of gel was cut off before autoradiography because the signals of the specific complexes were very weak compared with the signals from the free DNA. The results are presented so that the mobilities of the complexes are directly comparable with reference to the position of free DNA. Arrow head, specific DNA–protein complex; asterisk, non-specific complex in lysogen extract; F, free DNA; ○, *hex1*-specific complex; △, presumptive complex with two molecules of ASF-1 (ref. 13); W, wild-type sequence; M, mutant sequence. *b*, Binding site probes used in *a*. The −109 to −89 region of the *rbcS-3A* upstream fragment (−166 to −55) was substituted with the wild-type or mutant sequences (ref. 13 and E.L., unpublished data). The mutated bases are shaded. Lines with arrows indicate TGACG motifs or a similar motif, TGAGC. The numbers over the wild-type sequences show the nucleotide positions in the original promoters (wheat histone H3 promoter, CaMV 35S promoter, and T-DNA nopaline synthase promoter for *hex1*, *as-1*, and *nos1*, respectively (refs 6, 7 and 13).

METHODS. Poly(A)$^+$ RNA was prepared from leaves of tobacco (*Nicotiana tabacum* cv. SR1) adapted in the dark for two days[8]. A random-primed cDNA library was constructed in λgt11 vectors. The primary library was plated at a density of ~15,000 plaque-forming units per 15-cm plate. After 6-h incubation at 37 °C, IPTG-impregnated nitrocellulose filters were layered on top of the agar and the plates were further incubated at 37 °C for 6 h. The filters were processed at 4 °C by lifting and incubating them in B buffer (20 mM HEPES-KOH *p*H7.5, 40 mM KCl, 1 mM EDTA, 10% glycerol, 0.5 mM DTT) supplemented with 5% non-fat dry milk for 2 h. After a brief washing in B buffer the filters were incubated for 4 h in B buffer containing the labelled binding probe (2.5 ng ml^{-1}, 7×10^5 c.p.m. ml^{-1}) and 5 μg ml^{-1} sonicated and denatured salmon testis DNA. Synthetic double-stranded *hex1* oligonucleotides (21-bp sequence as shown in *b*) were concatemerized with T4 DNA ligase until the concatemers contained, on average eight copies of the sequence. The concatemers were labelled by nick translation and used as the binding probe. After incubation with the probe the filters were washed in B buffer for 50 min, briefly dried, and then autoradiographed. For gel retardation assays, lysogen extract and tobacco nuclear extract were prepared as described (refs 8 and 13). The probes were labelled at the *Hin*dIII sites (−166) by fill-in reaction with the Klenow fragment enzyme. After digestion of the labelled DNAs with *Bst*XI (−55), the probes were purified by polyacrylamide gel electrophoresis. The assay mixture contained lysogen extract (6.8 μg of protein) or tobacco extract (7.5 μg of protein), 0.1 ng of binding probe (4×10^4 c.p.m.), and 3 μg of poly(dI-dC) in 10 μl of B buffer supplemented with 0.8 mM phenylmethylsulphonyl fluoride. The mixture was incubated for 20 min at room temperature, before being loaded on to a non-denaturing 0.4% agarose–3% polyacrylamide composite gel. After electrophoresis, the gel was dried and autoradiographed with an intensifying screen. The exposure time was 12 h, except for the gel for hb2 that was exposed for 9 days after the free DNA portion was cut off.

To isolate cDNA clones encoding the DNA-binding proteins specific for the sequence TGACG, we screened a tobacco cDNA expression library using concatemers of *hex1* as a probe, as previously described[8,14]. Five positive clones were obtained from a primary library consisting of 6×10^4 recombinants. All five clones showed reproducible binding to *hex1*. The five λ clones, named hb1, -2, -3, -5, and -6, were divided into two groups according to the nucleotide sequences of their cDNA inserts. The protein encoded by the first group, which included hb1, -3, and -5, was named TGA1a and the protein encoded by the second group, which included hb2 and -6, was named TGA1b.

The binding specificities of TGA1a and TGA1b were confirmed by gel retardation assays with the lysogen extract of

hb1 and hb2, respectively (Fig. 1*a*). The lysogen extract of hb1 gives two DNA-protein complexes, one of them is specific to each of the wild-type probes, *hex1*, *as-1*, and *nos1*, but not to the mutant probes (Fig. 1*a*, marked by an arrow head). The other complex (marked by an asterisk in Fig. 1*a*) is also seen with the lysogen extract of the vector λgt11 and is, therefore, non-specific. These results demonstrate that TGA1a had the same specificity as ASF-1 for binding to these sequences. We consider TGA1a, therefore, to be a good candidate for ASF-1. The lysogen extract of hb2 gave four complexes specific only to the *hex1* wild-type probe (Fig. 1*a*, marked by arrow heads). These bands may represent different states of the complexes or proteolytic products. Because TGA1b bound only to *hex1*, TGA1b could correspond to the *hex1*-specific complex (marked by an open circle in Fig. 1*a*). The difference in binding specificity between TGA1a and TGA1b could be due to differences in the nucleotide sequence flanking the TGACG motif.

Sequence analysis of the inserts of hb1, -3, and -5, which encode TGA1a, shows that these clones seem to have been derived from the same messenger RNA species (Fig. 2*a*). The longest open reading frame (ORF) starting with a methionine is 1,077 bp, corresponding to 359 amino-acid residues with a relative molecular mass (M_r) of 40,694. Because no other significantly long ORF is found, we conclude that this ORF encodes TGA1a. The ORF in hb1 is out-of-frame from the reading frame of the *lacZ* gene. Surprisingly, in gel retardation assays the specific DNA-protein complex obtained with the lysogen extract of hb1 migrated similarly as that obtained with ASF-1 from tobacco extract (Fig. 1*a*). We presume that the cDNA insert had a cryptic sequence that functioned as a translation initiation site in *E. coli*. These results, as well as the results of northern blot analysis, indicate that the coding region of the cDNA insert in hb1 was near full-length. The deduced polypeptide has an acidic region (amino-acid residues 5–52; 12 acidic and 4 basic),

a

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1                GAATTCTTCAACGTACACCCAATTTGCTGCCTCAAGAAGG ATG GGT ATA TGC GAT CCG ATC CAT CAA CTT GGC ATG TGG GAT      82
1                                                         M   G   I   C   D   P   I   H   Q   L   G   M   W   D      14

 83  GAT TTC AAT AGT AGT TTC CCA AGT ACA TCG GCA ACC ATG ATT TTA GAA GTT GAT AAA TGC CTA GAG GAC CAG ATA CCA ATT ATG     166
 15   D   F   N   S   S   F   P   S   T   S   A   T   M   I   L   E   V   D   K   C   L   E   D   Q   I   P   I   M      42

167  GAG AAA AGA CTA GAC AAC GAG ACA GAA GAC ACT TCG CAT GGA ACA GTA GGG ACT TCT AAC AGA TAT GAA CCG GAA ACA AGT AAA     250
 43   E   K   R   L   D   N   E   T   E   D   T   S   H   G   T   V   G   T   S   N   R   Y   E   P   E   T   S   K      70

251  CCC GTC GAG AAG GTA CTT AGA CGT CTT GCA CAA AAC CGC GAG GCT GCT CGT AAA AGC CGT TTG CGG AAG AAG GCC TAT GTT CAG     334
 71   P   V   E   K   V   L   R   R   L   A   Q   N   R   E   A   A   R   K   S   R   L   R   K   K   A   Y   V   Q      98

335  CAG TTA GAA AAT AGT AAA TTG AAG CTG ATT CAA CTG GAA CAA GAA CTA GAA CGC GCC AGA AAA CAG GGC ATG TGT GTA GGT GGT     418
 99   Q   L   E   N   S   K   L   K   L   I   Q   L   E   Q   E   L   E   R   A   R   K   Q   G   M   C   V   G   G     126

419  GGT GTA GAT GCT AGC CAG CTA AGT TAC TCT GGA ACC GCT AGC TCA GGA ACT GCT GTA TTT GAT ATG GAG TAT GGT CAC TGG GTA     502
127   G   V   D   A   S   Q   L   S   Y   S   G   T   A   S   S   G   T   A   V   F   D   M   E   Y   G   H   W   V     154

503  GAA GAG CAA ACT AGA CAA ACA AAT GAC TTA AGG ATT GCT TTG CAT TCT CAA ATT GGT GAA GCG GAA TTG CGC ATT ATT GTT GAT     586
155   E   E   Q   T   R   Q   T   N   D   L   R   I   A   L   H   S   Q   I   G   E   A   E   L   R   I   I   V   D     182

587  GGT TAC CTG AAC CAC TAC TTT GAT CTC TTC CGC ATG AAA GCT ACG GCT GCT AAA GCT GAT GTC CTA TAC ATC ATG TCT GGT ATG     670
183   G   Y   L   N   H   Y   F   D   L   F   R   M   K   A   T   A   A   K   A   D   V   L   Y   I   M   S   G   M     210

671  TGG AAG ACA TCT GCC GAG CGC TTT TTC ATG TGG ATT GGA GGG TTT CGG CCA TCC GAG CTT CTA AAG GTT CTC ACA CCG CAT CTT     754
211   W   K   T   S   A   E   R   F   F   M   W   I   G   G   F   R   P   S   E   L   L   K   V   L   T   P   H   L     238

755  GAG CTC TTG ACA GAA CAA CAA CTT CGA GAG GTT TGT AAC CTG ACC CAA TCA TGT CAG CAA GCA GAA GAC GCC TTG TCA CAA GGA     838
239   E   L   L   T   E   Q   Q   L   R   E   V   C   N   L   T   Q   S   C   Q   Q   A   E   D   A   L   S   Q   G     266

839  ATG GTA AAA CTC CAC CAG ATT CTT GCC GAG GCT GTT GCA GCT GGC CGA CTA GGA GAA GGA AAT TAC ACT CTT CCG CAG ATG GGG     922
267   M   V   K   L   H   Q   I   L   A   E   A   V   A   A   G   R   L   G   E   G   N   Y   T   L   P   Q   M   G     294

923  CCT GCC ATC GAA AAG TTG GAA GAT CTT GTT AGG TTC GTA AAT CAG GCG GAT CAT CTA CGA CAA GAA ACC CTC CAA CAG ATG TCC    1006
295   P   A   I   E   K   L   E   D   L   V   R   F   V   N   Q   A   D   H   L   R   Q   E   T   L   Q   Q   M   S     322

1007 CGC ATC CTT AAT ACG TGC CAA GCA GCT CAG GGC TTA CTT GCC TTA GGG GAG TAC TTT GAA CGA CTT CGT GTT TTA AGC TCA CAA    1090
323   R   I   L   N   T   C   Q   A   A   Q   G   L   L   A   L   G   E   Y   F   E   R   L   R   V   L   S   S   Q     350

1091 TGG GCT ACT CGT CTA CGT GAG CCT ACC TAA TGA AGCACAAGAAGATCCGCTGTATATTACTCGAGGAGTTTTGCCTTCAGAAGATGATGCTGTGTTATGG    1190
351   W   A   T   R   L   R   E   P   T   *   *                                                                       359

1191 ACCAGAGTACTGTTGCTCACTTGGTATCTAAACCTATATAATCAGTGGCGGAGCCACACAGGTTCAAGGGCAGATGCAAATTCAGGATTCCAATGTTATTCGAATCTATTA    1301

1302 TTTGTACTTATTTACTGGATTTTAAACACATATATGTGATCTGAGCCAAAAACTACTAGGTTTGGATGAACCCATAAGTTATACACTAGATCGGCTCCTGCTCAAGGGTGT    1412

1413 TCAGTTGAGCATCCTTCGTCGGAAAATTATAGTGTGTTATATAAGTCAGATATTATGTGTTGAATCTTGAGCACACTTAGTGTAATTCTAGGCTTCGCCATTGAGCTATAT    1523

1524 TCATTCACTTCAGGTTTGTGGTGAATGAATTTTACCATCTTGCTACTTCTGGTAGGGTCTTGAGAACTTAAATGAGATTTTTACACAAATAGCC                    1617
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b

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  1  GAA TTC TGT GAT TTT TCC GGA AAT CAA GCA GCT GGA GGC GTT ATG GTT ATG GAT ACT TCA TCG CCG GAG CTT CGA CAG AGC TCA      84
  1   E   F   C   D   F   S   G   N   Q   A   A   G   G   V   M   V   M   D   T   S   S   P   E   L   R   Q   S   S      28

 85  AGC GGC TCA GAT GTT TTG AAT GCA ACG TCG TCG ACG TCG TCC CAC CAG GTT TCT GGC GAT GTC GCC GGG TAC CTG AAC GTG CCA     168
 29   S   G   S   D   V   L   N   A   T   S   S   T   S   S   H   Q   V   S   G   D   V   A   G   Y   L   N   V   P      56

169  TCG CCG GAG TCC AAT GGA TCC AAC CAT GAG GGT TCT CGG GAG TCT GCT AAT GAC AAC AAG GGT TTG GGT GAT GCT AGG GTT TTG     252
 57   S   P   E   S   N   G   S   N   H   E   G   S   R   E   S   A   N   D   N   K   G   L   G   D   A   R   V   L      84

253  AAT TGC CAT TCG CCG GAG TCG CAG GGT TCA GGC AAT TAT GGT TCA AAT GTC TCA GAA GGG CTG AAT TAT CCG TCA GAT TCG AAC     336
 85   N   C   H   S   P   E   S   Q   G   S   G   N   Y   G   S   N   V   S   E   G   L   N   Y   P   S   D   S   N     112

337  AAA TCG GTA CAT TCT TCT CCT AAT TTT GAA AAT AAT TCA ATA AAA AAT GGA GCT GTA GAA GAG AAA ATC AAA TTA GAG GGT GTC     420
113   K   S   V   H   S   S   P   N   F   E   N   N   S   I   K   N   G   A   V   E   E   K   I   K   L   E   G   V     140

421  AAT GCT AAT ATA AGT AAA TGT AGC TCC TTG TTG AAG AGG AAA AAA AGT AGT GAA GAT TCT AAT AAC ATA AAC ATA CAC CAA AAA     504
141   N   A   N   I   S   K   C   S   S   L   L   K   R   K   K   S   S   E   D   S   N   N   I   N   I   H   Q   K     168

505  TTG ACT AAT GTT GCA TTG AGT GAC AAT GTT AAT AAT GAT GAG GAT GAA AAG AAG AGA GCT AGA TTG GTT AGG AAT AGG GAA AGT     588
169   L   T   N   V   A   L   S   D   N   V   N   N   D   E   D   E   K   K   R   A   R   L   V   R   N   R   E   S     196

589  GCT CAA CTG TCA AGG CAA AGA AAG AAG CAC TAT GTT GAG GAA TTA GAA GAT AAA GTT AGA ATA ATG CAT TCA ACA ATT CAA GAT     672
197   A   Q   L   S   R   Q   R   K   K   H   Y   V   E   E   L   E   D   K   V   R   I   M   H   S   T   I   Q   D     224

673  TTG AAT GCT AAG GTA GCT TAT ATA ATT GCG GAA AAT GCT ACT CTA AAG ACG CAG                                          726
225   L   N   A   K   V   A   Y   I   I   A   E   N   A   T   L   K   T   Q                                          242
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c

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                                                  *   *   *   *   *   Binding site sequence
C-Jun    (259-317)  QERIKAERKRMRNRIAASKCRKRKLERIARLEEKVKTLKAQNSELASTANMLREQVAQL          ATGAC TCT
GCN4     (223-281)  ESSDPAALKRARNTEAARRSRARKLQRMKQLEDKVEELLSKNYHLENEVARLKKLVGER          TGAC TCA
CREB     (266-324)  EAARKREVRLMKNREAARECRRKKKEYVKCLENRMAVLENQNKTLIEELKALKDLYCHK          TGACGTCA

TGA1a    ( 70-128)  KPVEKVLERLAQNREAARKSRLRKKAYVQQLENSKLKLIQLEQELERARKQGMCVGGGV          TGACG
TGA1b    (181-239)  DEDEKKRRRLMRNRESAQISRQRKKEYVSELEDKVRIMHSTIQDLNAKVAYIIAENAIL          TGACG
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FIG. 2 DNA and deduced amino acid sequences of TGA1a and TGA1b. *a*, DNA sequence of the cDNA insert in hb5. The deduced amino acid sequence for the longest ORF is shown below the DNA sequence in the single-letter code. The first methionine residue (M) in the figure, assumed to be the translation initiation site, is numbered as 1. Hb1 and -3 contained insert DNA sequences corresponding to nucleotide numbers 1–1,262 and 1–1,276, respectively. *b*, DNA sequence of the cDNA insert of hb2. The deduced amino acid sequence is shown as *a*. The first glutamate residue (E) in the insert is numbered as 1 for convenience. Hb6 contained insert DNA sequence very closely related to that of hb2 nucleotide-numbers 1–676 (~95% homology). *c*, Amino-acid residues 70–128 of TGA1a and amino-acid residues 181–239 of TGA1b are compared with homologous regions of c-Jun, GCN4, and CREB[1–4]. The positions of the homologous regions are indicated in parentheses as amino-acid residue numbers. The binding-site sequences for the five DNA-binding proteins are also indicated. Conserved residues between TGA1a, TGA1b, and the other three proteins are enclosed by thin lines, whereas the residues conserved only among TGA1a, TGA1b, and CREB are enclosed with bold lines. Asterisks indicate leucine residues for the leucine-zipper.

METHODS. The cDNA inserts of hb1, -2, -3 and -5 were subcloned as restriction fragments into M13mp18 and M13mp19 vectors. Nucleotide sequences of both strands were determined by Sequenase™ sequencing kit (USB) with common primers and synthesized primers. To obtain overlaps between some subclones, double-stranded DNA sequencing was carried out using the pUC sequencing kit (Boehringer). Sequence data were processed by DNASIS and PROSIS programs (Hitachi) on an IBM PS/2 computer. The homologies shown in *c* were detected by visual inspection.

a basic region (amino-acid residues 70–94; 11 basic and 2 acidic), a putative leucine-zipper region[5] (leucine residues 100, 107, and 114), and a relatively glutamine-rich region (amino-acid residues 244–332; 14 glutamine residues). The acidic region could be involved in activation of transcription, because acidic domains function as activating regions in several DNA-binding proteins[9].

Figure 2*b* shows the DNA sequence of the insert of hb2, which encodes TGA1b, and the deduced amino acid sequence for the longest ORF. The ORF is in-frame with the reading frame of *lacZ*. Although this was not a full-length clone, the deduced polypeptide has some interesting characteristics. It contains a serine-rich region (amino-acid residues 20–160; 33 serine residues), a basic region (residues 185–206; 11 basic residues and 1 acidic residue), and a putative leucine-zipper region (leucine, methionine, or isoleucine residues at positions 211, 218, 225, 232, and 239). There is no significant homology between TGA1a and TGA1b other than the basic region and the leucine-zipper region.

The basic regions of TGA1a and TGA1b are homologous to those in the DNA-binding domains of CREB, GCN4, and c-Jun[1–4] (Fig. 2*c*). The binding site of CREB contains a TGACG motif, whereas the binding sites of GCN4 and c-Jun contain a similar TGACT motif[1–4]. Among the three, CREB has the highest homology to the basic region in TGA1a and TGA1b. It seems reasonable to predict that the amino-acid residues that are conserved only among CREB, TGA1a, and TGA1b (enclosed by bold lines in Fig. 2*c*) are important in distinguishing the TGACG motif from the TGACT motif. This prediction could be tested by site-directed mutagenesis. The nuclear factors CREB, GCN4, and c-Jun are members of a class of DNA binding proteins that contain a leucine-zipper region[6]. This region is at the carboxy-terminal end of the basic region. When the amino-acid sequences of these proteins are aligned according to their homology in the basic region, the first three of the four leucine residues (marked by asterisks in Fig. 2*c*) of the leucine zipper in CREB, GCN4, and c-Jun are conserved in TGA1a. In TGA1b, residues in the corresponding positions are Leu, Met, Leu, Ile and Leu. Because a leucine residue in the leucine-zipper of the heat-stable DNA-binding protein C/EBP can be substituted by a methionine or isoleucine residue (although with some reduction in the potential of the protein to form dimers)[11], we consider this region of TGA1b to be a variant of the leucine-zipper. Such a high degree of conservation among these proteins that come from distantly related organisms (human, yeast, and higher plant), suggest that these leucine residues are functionally equivalent.

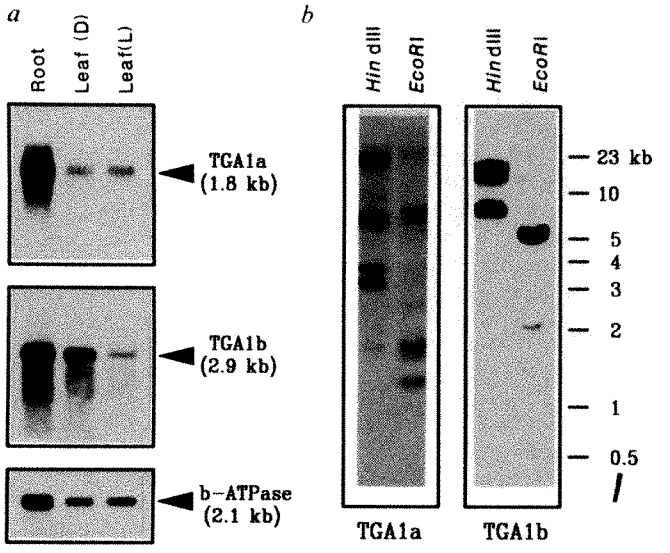

FIG. 3 Northern and Southern blot analyses of TGA1a and TGA1b genes, *a*, Northern blot analysis of RNA from tobacco leaves and roots with TGA1a and TGA1b genes. Each lane contained 2 μg of poly(A)$^+$ RNA. Messenger RNA sizes were determined by comparison with molecular size markers. Leaf(D), leaves from tobacco plants kept in the dark for two days; Leaf(L), leaves from light-grown tobacco plants: Root, roots from light-grown tobacco plants. *b*, Southern blot analysis of TGA1a and TGA1b. Each lane contained 17 μg of tobacco DNA digested with either *Hin*dIII or *Eco*RI. Positions of molecular size markers are indicated on the right.
METHODS. Preparation of RNA and DNA samples from *Nicotiana tabacum* cv. SR1 and northern and Southern blot analysis were as described.[8] The 1,150-bp *Eco*RI-*Xho*I fragment from hb1, the 560-bp *Eco*RI-*Fok*I fragment from hb2, and the 1.3-kb *Hin*dIII-*Xba*I fragment from the b-ATPase gene *atp2-1* (ref. 11) were labelled by random primed DNA labelling kit (Boehringer) and used as the TGA1a, the TGA1b, and the b-ATPase probe, respectively. The specific activities of the probes were $\sim 1.8 \times 10^9$ c.p.m. per μg. The same filter was sequentially hybridized with TGA1a, TGA1b, and b-ATPase probes, after washing off the previous probe by boiling in water. Filters were pre-hybridized and hybridized in a buffer containing 50% formamide, 10% dextran sulphate, 25 μg ml^{-1} sonicated and denatured salmon testis DNA, 1 × Denhardt's solution, and 5 × SSC at 43 °C. About 4×10^6 c.p.m. ml^{-1} of probes were used. The filters were washed in 2 × SSC, 0.1% SDS and then in 0.1 × SSC, 0.1% SDS at room temperature. They were washed finally in 0.1 × SSC, 0.1% SDS at 50 °C, briefly dried, and then autoradiographed with an intensifying screen. The exposure times for TGA1a, TGA1b, and b-ATPase in *a* were 3 h, 1 day, and 1 h, respectively. The exposure times for TGA1a and TGA1b in *b* were 7 h and 10 h, respectively.

Northern blot analysis of tobacco RNA with the TGA1a cDNA insert showed that the level of TGA1a mRNA (1.8 kb) in roots was ~10 times higher than it was in light-grown or dark-adapted leaves (Fig. 3*a*). The level of TGA1b mRNA (2.9 kb) in roots was two- to fivefold higher than it was in leaves; dark-adapted leaves contained twice as much TGA1b mRNA as light-grown ones (Fig. 3*a*). As a control, we found that the expression of the beta subunit of mitochondrial ATP synthase (b-ATPase) was about twofold higher in roots than it was in leaves, confirming previous results (Fig. 3*a*)[11]. The finding of much higher expression of TGA1a in roots than in leaves is correlated with the observation that *as-1* is involved in the root expression of the 35S promoter[13] and is consistent with the proposal that the functional form of ASF-1 could be limiting in leaves.

Southern blot analyses of the genes encoding TGA1a and TGA1b showed only a few bands under relatively stringent conditions of hybridization (Fig. 3*b*). Under these conditions, the two genes did not cross-hybridize. Thus, it is likely that each of them is encoded by a small gene family. Because northern blot analyses gave only a single band for each gene (Fig. 3*a*), both gene families probably contain very closely related members.

The high homology in the DNA-binding domains among TGA1a, TGA1b, and CREB suggests that they have an ancient origin, because plants and animals diverged $\sim 1.5 \times 10^9$ years ago[12]. Analysis of the TGA1a and TGA1b genomic clones will reveal whether this DNA-binding domain is encoded by a separate exon. Moreover, the extent of homology suggests possible conservation in functions other than their DNA binding specificity. In mammalian systems, CREB is involved in signal transduction pathways through kinases, notably protein kinase A. The proteins TGA1a and TGA1b may be inolved in similar signal transduction pathways in higher plants. □

Received 31 March; accepted 19 July 1989.

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ACKNOWLEDGEMENTS. We thank Karen LaMarco and Steven McKnight for their DNA-binding protein-encoding λ clone that we used as a positive control for our screening. We thank Bettina Niner and Hugh Williams for oligonucleotide synthesis and photography, respectively, and Philip Benfey for helpful discussion. E.L. was supported by an NIH post-doctoral fellowship. This work was supported by a grant from Monsanto Co.

Three-dimensional structure of *Escherichia coli* RNA polymerase holoenzyme determined by electron crystallography

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DURING transcription in *E. coli*, the DNA-dependent RNA polymerase locates specific promoter sequences in the DNA template, melts a small region containing the transcription start site, initiates RNA synthesis, processively elongates the transcript, and finally terminates and releases the RNA product. Each step is regulated by interactions between the polymerase, the DNA, the nascent RNA, and a variety of regulatory proteins and ligands[1-3]. The *E. coli* enzyme contains a catalytic core of two α-subunits, one β- and one β′-subunit, with relative molecular masses (M_r) of 36,512, 150,619 and 155,162, respectively[2]. The holoenzyme has an additional regulatory subunit, normally σ_{70}, of M_r 70,236. Preparations may also contain the ω-subunit ($M_r \sim 10{,}000$), which can be removed without affecting any known properties of the enzyme[2]. Because the amino-acid sequences of the β- and β′-subunits are homologous to those of the largest subunits of the yeast, *Drosophila* and murine RNA polymerases[4-7], it seems likely that essential features of the three-dimensional structure and catalytic mechanism of RNA polymerase are also conserved across species. Crystals of RNA polymerase suitable for X-ray analysis have not yet been obtained, but two-dimensional crystals of *E. coli* RNA polymerase holoenzyme can be grown on positively charged lipid layers[8]. Electron microscopy of these crystals in negative stain shows the enzyme in projection as an irregularly shaped complex ~100 × 100 × 160 Å in size. We have now determined the three-dimensional structure by electron microscopy of negatively stained, two-dimensional crystals tilted at various angles to the incident electron beam[9]. We find a structure in RNA polymerase similar to the active-site cleft of DNA polymerase I (ref. 10). In the light of functional similarities between these two enzymes, together with other evidence, this probably identifies the active-site region of RNA polymerase.

Two-dimensional crystals of *E. coli* RNA polymerase holoenzyme were formed on layers of positively charged lipid, transferred to an electron microscope grid, and negatively stained with 1% uranyl acetate as described previously[8]. The crystals contained holoenzyme, and not simply core enzyme that had lost the σ_{70} subunit during the crystallization process, as monoclonal antibodies to the σ_{70} subunit[11] bound specifically to the crystallized RNA polymerase (shown by electron microscopy; S.A.D., N. E. Thompson and R. R. Burgess, data not shown). Furthermore, purified core enzyme failed to crystallize under the same conditions. Presumably, the crystals also contained the ω-subunit, as this polypeptide was present in roughly stoichiometric amounts in the sample used for crystallization (D. N. Arnosti and M. J. Chamberlin, personal communication). Crystalline areas ranged in size from 0.2 to 0.7 μm^2 and were ~100 Å thick[8]. The array can be described as a crystal of space group P2 ($a = 201$ Å, $b = 200$ Å, $\gamma = 107°$) with a thickness of one unit cell in the direction of the c axis. Thus, each crystalline area contained 500-2,000 unit cells or 1,000-4,000 molecules.

Three micrographs were recorded from each crystalline area using low electron doses (<10 electrons per Å^2) at a calibrated magnification of ×27,800, two with the specimen tilted to the incident 100 kV electron beam, and one untilted. Micrographs of test areas showed no noticeable degradation in the diffraction data after three exposures, even for the highest resolution spots observed. Micrographs of similar quality were selected, digitized, and processed by computer using standard methods[9]. Additional computations incorporated procedures for correcting lattice distortions by reciprocal space filtering, real space correlation analysis, and spline function interpolation, as described by Henderson *et al.*[12].

A total of 74 micrographs of crystals tilted 0-80° to the incident electron beam were used. The average phase error, based on refinement of each new measurement with all previously accumulated symmetry-related phases within 0.00227 Å^{-1} in z^*, was 14° when the data were combined according to the crystallographic space group P2. No single micrograph had an average phase error greater than 20°. Refinements were also performed to investigate the possibility of 2-fold or screw axes in the plane of the lattice. The minimum average phase error was 39° in each case. A refinement of the data was also performed assuming no symmetry (space group P1). The resulting structure showed two regions of protein density per unit cell, both very similar in appearance to each other and to the final structure determined assuming P2 symmetry, and strongly related by a 2-fold axis perpendicular to the lipid layer. No other symmetry elements were apparent. These results and the excellent refinement of the data assuming P2 symmetry strongly supported the choice of space group. Finally, either molecule in the unit cell of the P1 structure was consistent with the conclusions of this work.

A least-squares procedure[13] was used to fit smooth curves (Fig. 1) to the combined amplitude and phase data for each of the 93 crystallographically independent lattice lines included in the reconstruction. By sampling these curves at intervals of 0.00333 Å^{-1} in z^*, 552 Fourier terms were collected and used to calculate the three-dimensional map. Except for the cone of reciprocal space not sampled because of the upper limit on tilt angles, the data were nearly complete to a resolution of ~27 Å, with a considerable amount of information beyond that resolution (Fig. 2). To help assess the effect of the missing data, edge-on views of the crystals[8] were analysed to give directly the variations in amplitude and phase along the 1, 1 lattice line. Estimates of the variations along the 0, 0 lattice line were also obtained from the edge-on views, but this information was obscured by noise near the origin of the transforms because of uneven stain distribution across the lipid layer. The data from edge-on views for the 1, 1 lattice line were adjusted to give the best fit to the 1, 1 data from the tilt experiments (Fig. 1). The amplitude scale factor and phase origin adjustment needed to fit the 1, 1 data were used to correct the estimated variations along the 0, 0 lattice line. It was found that the basic features of the three-dimensional map were not affected by including estimates of amplitude and phase along the 0, 0 lattice line. Thus, owing to the poor quality of the 0, 0 lattice line data, they were not included in the final reconstruction presented here.

The map calculated for RNA polymerase in negative stain shows the surface topography of the molecule, so the outermost contour is of interest. This contour was chosen such that the individual molecules in the array were completely disconnected. The overall dimensions of a protein molecule were then ~90 × 95 × 160 Å, consistent with the dimensions determined previously by analysis in projection and from views of single particles[8,14]. Assuming a protein density of 1.3 g cm^3, the volume occupied by a molecule corresponds to an $M_r \sim 370{,}000$, about 82% of that expected for the RNA polymerase holoenzyme

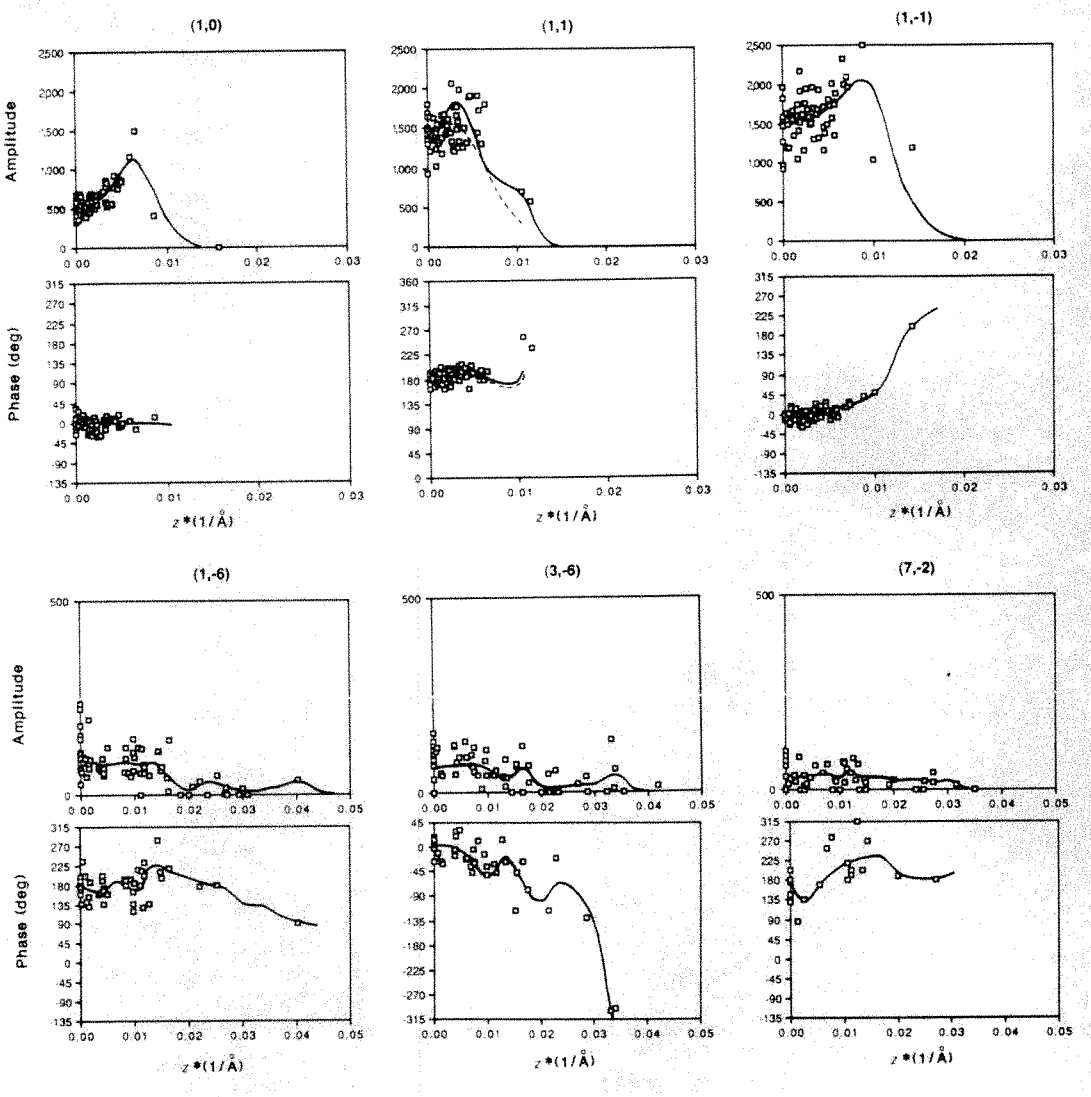

FIG. 1 Values of amplitude and phase determined along reciprocal lattice lines from micrographs of specimens tilted to the incident electron beam at angles 0–80°; z^* is the distance in reciprocal space in the direction of the c^* axis. The tilt range 0–55° was obtained using a goniometer stage; additional tilts up to 80° were obtained by bending the specimen grids. The data have been combined, assuming the space group P2. The smooth curves (solid lines) drawn through the data were determined by a least-squares procedure[13]. Only phase data from reflections with amplitudes greater than twice background were plotted and used in the curve fitting. Shown are three of the strongest amplitude lattice lines (1, 0; 1, 1; 1, −1) and three lattice lines near the resolution limit of ~27 Å (1, −6; 3, −6; 7, −2). Shown along with the data for the 1, 1 lattice line are the data obtained from edge-on views (broken lines), which were adjusted to give the best fit to the tilt data.

(~450,000)[2]. The small discrepancy is probably mainly due to some penetration of the RNA polymerase molecule by negative stain, which is excluded from hydrophobic regions but not necessarily by the outer surfaces of proteins[9].

The most striking feature of the map is a thumb-like projection that surrounds a cylindrical channel. Viewing the map parallel to the plane of the lipid layer (Fig. 3*a*), one looks towards the front of the channel, whereas viewing the map at an angle from beneath the lipid layer (Fig. 3*b*), one sights along the axis of the channel. The channel is ~25 Å in diameter and 55 Å in length, and is thus of appropriate dimensions for binding double-helical DNA. Support for this possible role comes from a remarkable similarity between the channel and the proposed DNA-binding-site cleft in the X-ray crystal structure of the large (Klenow) fragment of *E. coli* DNA polymerase I (ref. 10). Figure 3 illustrates the good fit of size and shape in the region of the cleft between the RNA polymerase map and the α-carbon backbone of DNA polymerase. An apparent deviation occurs at the tip of the thumb-like projection of the RNA polymerase map, where there is a region of density not matched by any structural features in the DNA polymerase α-carbon backbone. At just this point in the DNA polymerase structure, however, there is a stretch of 50 residues that are not resolved because of disorder or motion in the crystal. The α-carbon atoms on each side of the unobserved gap are indicated in red (Fig. 3). The position of the extra density in the RNA polymerase map is clearly consistent with it being the structural counterpart of

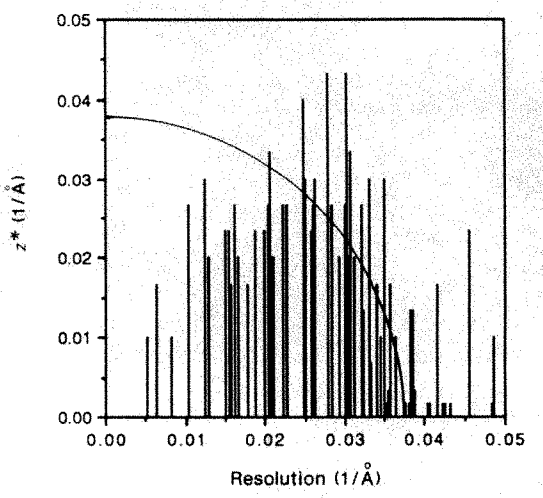

FIG. 2 Schematic diagram showing the region of reciprocal space used in the reconstruction. The 27-Å resolution sphere is included.

the flexible domain in the DNA polymerase structure. The low resolution of the RNA polymerase structure (compared with that of the X-ray structure of DNA polymerase) allows this feature to be observed. The existence in DNA and RNA polymerases of this flexible domain, which might surround the DNA substrate, suggests a means by which these enzymes could act processively in the polymerization of their respective products[10,15].

The significance of the structural similarity between DNA and RNA polymerases is supported by a small degree of amino-acid sequence conservation[4]. There is a weak homology between residues 666–695 in DNA polymerase I and residues 350–380 of the β-subunit of RNA polymerase[4]. The conserved residues are on the floor of the DNA-binding cleft of DNA polymerase I (ref. 10), part of the region that matches well with the RNA polymerase structure. Moreover, the β'-subunit of RNA polymerase is believed, based on other grounds, to be involved in DNA binding[1].

The 55 Å length of the putative DNA-binding channel in the RNA polymerase map is sufficient to accommodate ~16 base pairs of double-helical DNA in the B form, which may relate to the 16–18 base pairs that become unwound in the transcription complex[16]. This single-stranded region lies within a longer stretch of 50–60 base pairs (at least 170-Å long in the B form) associated with the enzyme in the open-promoter complex[17]. There does not seem to be any way in which a 170 Å length of DNA can be bound to the enzyme without significant bending of the DNA. Evidence for such bending has come from electrophoretic-mobility shift experiments[18]. The path followed by the DNA across the surface of the enzyme remains to be seen. Crystallization and imaging of enzyme–DNA complexes by the methods used here should help assess the role of the putative DNA-binding channel and reveal other features of the protein–DNA interaction. □

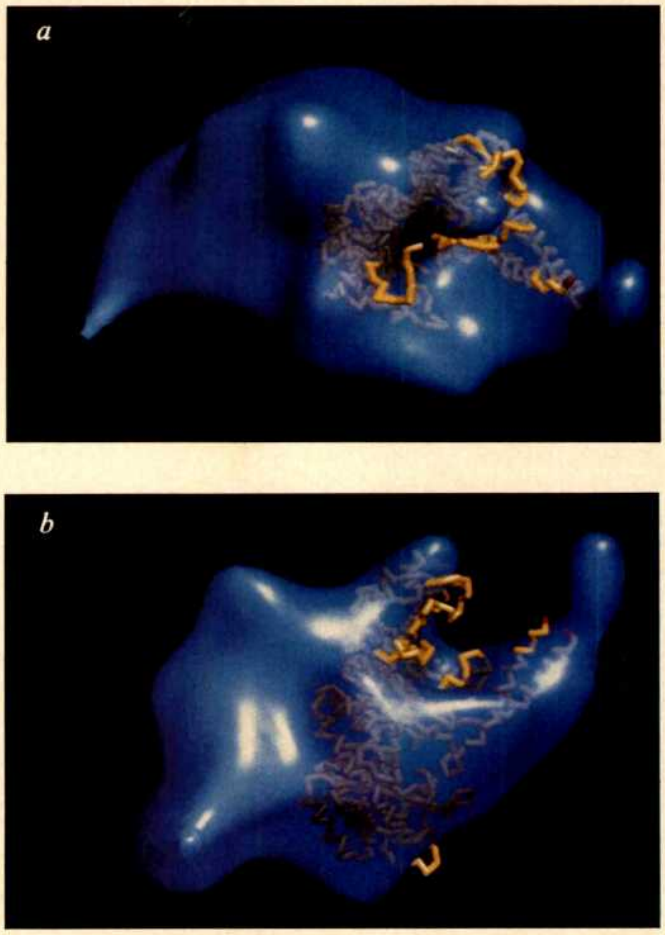

FIG. 3 Computer-generated models of a single *E. coli* RNA polymerase holoenzyme molecule. Matched to the RNA polymerase map is the α-carbon backbone of the DNA polymerase I Klenow fragment[10] (shown in grey, except for parts protruding from the RNA polymerase map, which are shown in yellow). The α-carbon atoms on each side of the unobserved gap in the DNA polymerase chain[10] are shown in red. The contour represents negative stain-excluding region. *a*, The molecule viewed in the plane of the two-dimensional array and perpendicular to the crystallographic *a*-axis. The lipid layer to which the molecule is adsorbed would lie underneath. *b*, The molecule viewed from the bottom (as if looking up through the lipid layer) along the axis of the cleft. Images courtesy of D. S. Goodsell and A. J. Olson[19] (magnification, $\times 4.7 \times 10^6$).

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ACKNOWLEDGEMENTS. We thank Dr M. J. Chamberlin and the members of his laboratory for providing pure RNA polymerase and for suggestions and encouragement. We also thank Drs C. W. Akey, H. O. Ribi and P. N. T. Unwin for discussion. S.A.D. was a reclpient of an American Cancer Society Postdoctoral Fellowship. This work was supported by the NIH.

ERRATA

New light on the Lysenko era

Valery N. Soyfer

Nature **339,** 415–420 (1989)

IN this Commentary, the author's affiliation (page 420) should read: Department of Molecular Genetics and Biotechnology Center, The Ohio State University, Columbus, Ohio 43210, USA. And the sentence running from page 417 to page 418 should read: "Lysenko had also harmed his own cause by his resolute repudiation of plant hormones, and his reputation was undermined when the Dutchman Went and the Soviet psychologist N. G. Kholodony were honoured for their discovery." □

Substrate specificity and affinity of a protein modulated by bound water molecules

F. A. Quiocho, D. K. Wilson & N. K. Vyas

Nature **340,** 404–407 (1989)

THE 'Acknowledgements' section was omitted from the above letter during the production process. It should read: This work was supported by the Howard Hughes Medical Institute and grants from NIH and the Welch Foundation. □

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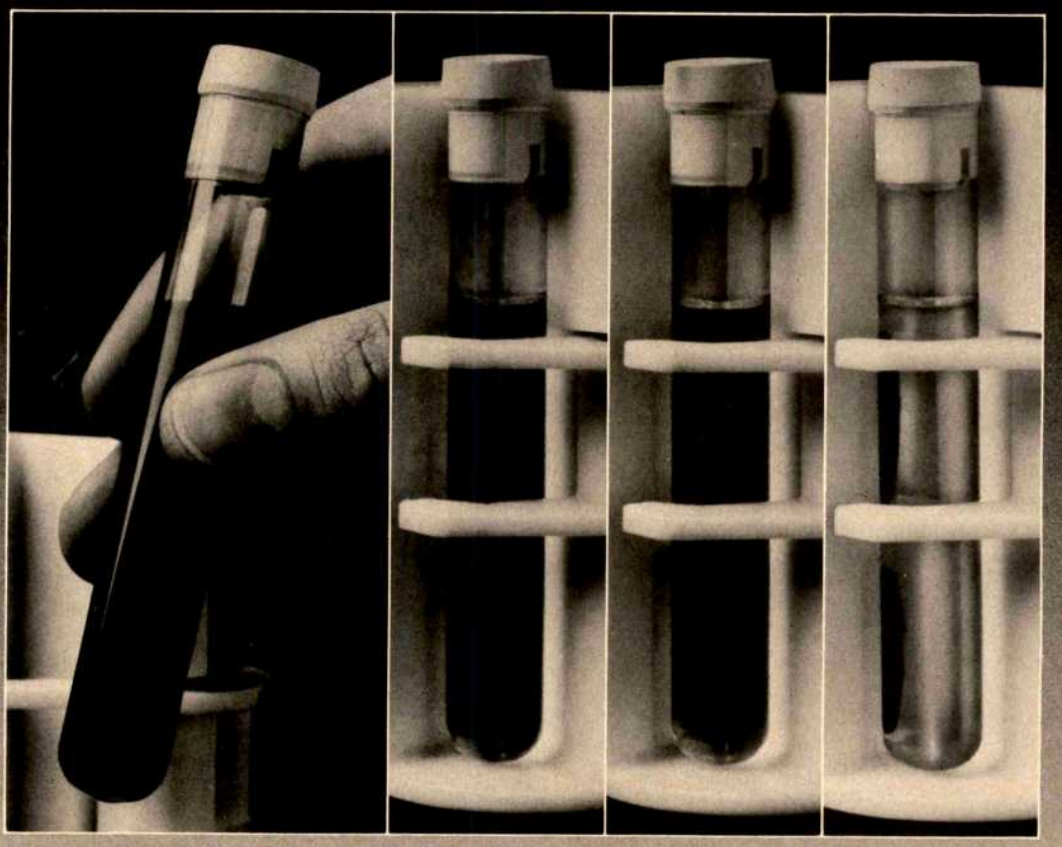

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Magnetic separation of DNA

M. Uhlen

Originally developed for immunoassays, magnetic beads in combination with streptavidin-biotin technology have demonstrated their power for separating DNA and RNA.

SOLID-phase methods have proven to be very useful for the separation, synthesis and diagnostic detection of biomolecules. The solid-phase approach produces reproducible reactions with high yields, and allows solutions to be changed rapidly. Routine methods suitable for automation can be designed relatively easily using a solid phase, because of the ease with which it can be separated from the reaction solution.

The solid phase may consist of the walls of test tubes or microtitre wells, or polymer particles (such as agarose or silica) packed in columns. Magnetic particles can also be used as a solid support for the separation of non-clarified cell suspensions or lysates without the need for centrifugation or filtration. If the magnetic beads are non-porous, adsorption and desorption of biomolecules occur at the surface, providing reaction kinetics similar to those found in free solution.

Attractive separations

Early magnetic particles, made by the polymerization of acrylamide and agarose with paramagnetic materials[1], were heterogeneous in size and magnetite content. Hydrophilic beads have now been developed that are identical in size, density and amount of magnetized material[2]. Such beads sediment homogeneously in magnetic fields, and separations using them can often be accomplished within a few seconds. The chemical structure of the particle surface may be varied[3], providing a flexible system for the immobilization of biomolecules.

Immunomagnetic separations, using immobilized monoclonal and polyclonal antibodies, have been described where the magnetic beads are directed to targets such as cells, organelles or macromolecules. The speed of magnetic separation is thus combined with the specificity of antibodies. Immunological applications based on this concept have been developed for tissue typing and cancer therapy, as well as for the selection of monoclonal antibody-producing cells and the separation of subcellular components[4].

Biotin-streptavidin

Magnetic separation can also be used as a tool in molecular biology, by coupling specific DNA fragments to the beads and using them as probes or templates. The fragments can be bound to the beads through a reactive amino or thiol group on a synthesized oligonucleotide, using the procedures developed for the immobilization of antibodies. But a more versatile system uses magnetic particles covalently bound to streptavidin, for the directed immobilization of both double-stranded and single-stranded biotinylated DNA. The remarkable stability and strength ($K_d = 10^{-15}$) of the non-covalent biotin–streptavidin interaction allows DNA manipulation reactions, such as strand melting, elution and hybridization (using alkali, temperature or formamide), to be performed without interfering with the binding of the DNA to the beads[5].

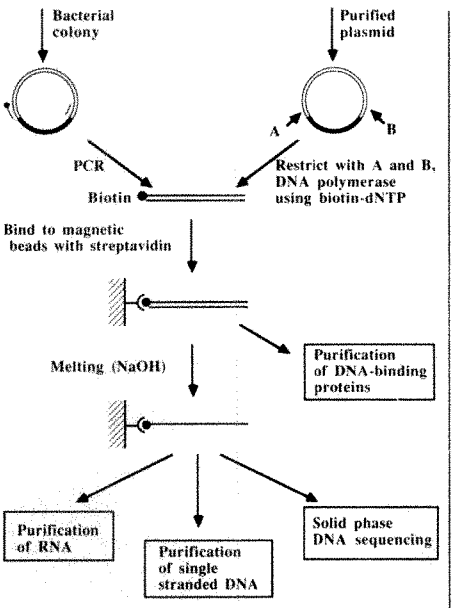

FIG. 1 Possible applications for DNA–containing magnetic beads in molecular biology.

The DNA-bead complex is also resistant to high concentrations of urea and salt. Several alternative strategies exist for the selective introduction of biotin into one of the strands of the DNA. The most straightforward approach is to synthesize an oligonucleotide containing a chemically introduced biotin molecule[6], and then directly immobilizing the oligonucleotide onto the streptavidin-coated bead. An even more flexible approach (Fig. 1) is to perform endonuclease restriction of a purified plasmid, followed by a fill-in reaction with DNA polymerase using one or several biotinylated nucleotides[5]. Alternatively, a polymerase chain reaction (PCR)[7] can be performed using thermostable DNA polymerases such as *Taq* or *Tth* and a biotinylated oligonucleotide[8]. The latter two procedures can immobilize cloned fragments in sizes ranging from a few nucleotides to several kilobases. The techniques also yield immobilized double-stranded DNA, which may be converted into single-stranded DNA through an appropriate melting procedure[5,8].

Molecular applications

The use of magnetic beads in combination with the biotin–streptavidin system has allowed the direct solid-phase sequencing of both genomic and plasmid DNA[8]. The system allows the *in vitro* amplification and sequencing reactions to be performed under optimal conditions because the buffers and enzymes can be changed easily. A protocol has been described for the direct sequencing of plasmid DNA starting from a single bacterial colony, without the need for previous template purification[8].

The rapid magnetic system can also be substituted for conventional affinity chromatography for the purification of DNA-binding proteins. Three cycles of adsorption and desorption to magnetic beads containing a specific double-stranded recognition sequence has yielded a nearly homogeneous nuclear factor (IIIC) in less than one hour from a yeast crude extract[9].

Beads containing single-stranded DNA can be used for the separation and isolation of RNA and single-stranded DNA. Using monosized beads with coupled oligo(dT)$_{36}$, mRNA has been purified with a high yield from a crude extract of a mouse hybridoma cell line within two minutes[10]. The low number of RNA handling steps and the short time required to perform the technique reduce the risk of physical and enzymatic degradation of the RNA. It is also possible to isolate specific RNA molecules using cloned or synthesized DNA fragments as probes. Obviously, the RNA molecules recovered on the magnetic beads can subsequently be used for solid-phase cDNA synthesis.

The magnetic particles can also be used to recover specific single-stranded DNA fragments from a complex mixture of fragments[11]. New cloning strategies can be envisioned where DNA fragments obtained from plasmid, lambda, cosmid or genomic DNA could be specifically recovered by the magnetic beads and subsequently amplified using PCR. In this way, chromosome walking and other operations could be achieved without the need for subcloning.

Finally, the magnetic solid-phase technology can be used for separation steps during the analysis of specific DNA or RNA sequences. Such an assay has been described which uses a DNA probe-based hybridization reaction for the detection of HIV-1-specific RNA in infected cells[12]. Specific hybridization complexes can be purified using a magnetic approach called target cycling/background reduction, in which hybrids are captured from solution with a complementary sequence attached to magnetic particles. We have recently used magnetic beads in an assay for the detection of immobilized amplified nucleic acids, in which samples amplified using a two-step PCR are directly captured using the biotin–streptavidin system. The amount of immobilized amplified fragment can then be detected using an isotope or colourimetric procedure. The assay is rapid and might — in some applications — eliminate the need for electrophoresis, restriction mapping or hybridization assays in the analysis of PCR-amplified materials. □

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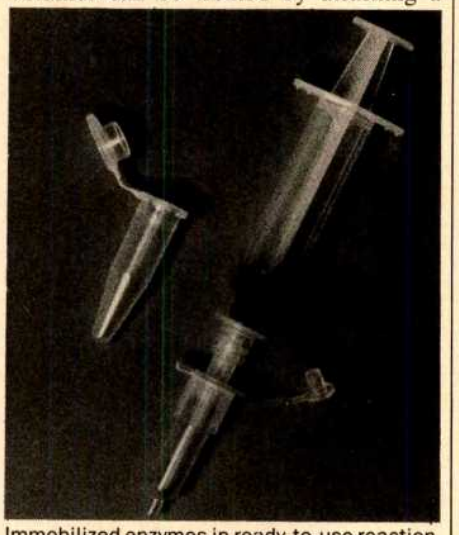
Separation systems

This week features a range of products to help with difficult separations, from isoelectric focusing to the concentration of shear-sensitive cells.

MOBITEC says its **immobilized enzymes** in compact, reusable reaction columns are ideal for enzymatic reactions involving small substrate volumes (*Reader Service No. 101*). The immobilized enzymes available include specific and nonspecific proteases, DNase I, RNases, lipases, esterases, oxidases, alkaline phosphatase and β-galactosidase. The enzymes are covalently bound to agarose using "elastic" linkers, which Mobitec says achieves high enzyme density without reducing enzyme activity. Up to 100 μl of substrate can be applied to the column: larger volumes can be treated by attaching a standard syringe. The enzyme-treated substrate is then spun out of the column while the enzyme is retained. Mobitec says enzymes are more stable in an immobilized form than in solution, and can be stored at 4 °C between use.



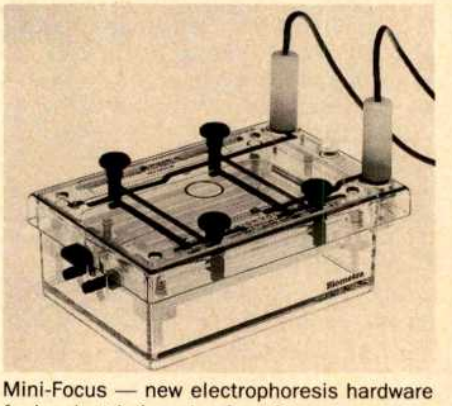
Immobilized enzymes in ready-to-use reaction columns from Mobitec.

Free copies of Bio-Rad's 1989 Chemical Division **catalogue** are now available (*Reader Service No. 102*). The 200-page colour catalogue features new products for chromatography, HPLC, electrophoresis, immunochemistry, molecular biology, and liquid handling. New ideas for chromatography include chromatography gels and resins that are free of endo- and exonucleases, ion exchange membranes as an alternative to column chromatography, prepacked Econo-Pac 10 columns for MAb and IgG purification, and fraction collectors for use in coldroom operations. The section on HPLC offers a new line of isocratic and gradient HRLC systems, columns for hydrophobic interaction chromatography, and a programmable refrigerated autosampler. New items for electrophoresis include a high-performance capillary electrophoresis system, which Bio-Rad says combines the high resolution of electrophoresis with the rapid on-line detection of HPLC, and a semi-dry electrophoretic blotting cell. The CHEF-DR megabase DNA pulsed field electrophoresis system is new in the molecular biology section.

For achieving high filtration rates without clogging, Bio-Recovery, Inc. recommends its X-FLO tangential flow **cell harvesting system** (*Reader Service No. 103*). The membrane-based system has an open-channel design, which the company says can concentrate 20 litres to 200 millilitres with flux rates of 200–400 LMH. The system can continue to function even at solid levels of 40 per cent dry weight, says Bio-Recovery. When operations are scaled up for processing 500- and 5,000-litre batches, Bio-Recovery says results are accurate to ± 5 and 10 per cent, respectively. X-FLO systems are available in sanitary, autoclavable and solvent-resistant designs.

Electrophoresis essentials

Mini-Focus is the name of the new gel apparatus from Biometra for **flat bed isoelectric focusing** using either polyacrylamide or agarose gels (*Reader Service No. 104*). The electrodes — made of synthetic carbon — are placed in direct contact with the gel surface, eliminating the need for extra buffers and wicks. Biometra says this arrangement produces a uniform electric field and results in sharp bands and high reproducibility. The £645 (UK) gel apparatus comes complete with condensation shield, height-adjustable cooling device and electrodes. The gel chamber can accommodate either analytical or preparative gels on gel bond foils that



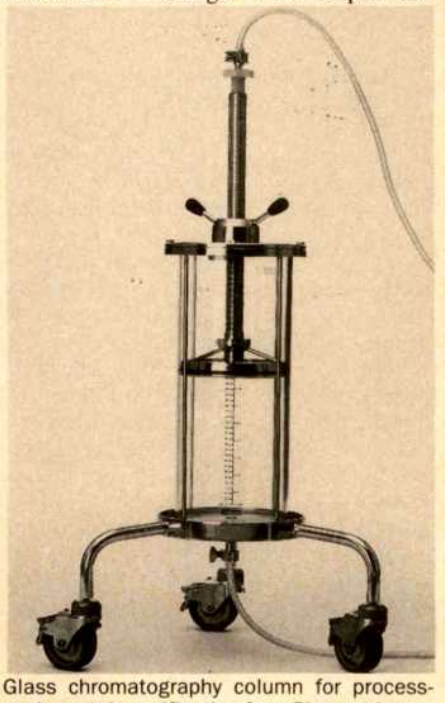
Mini-Focus — new electrophoresis hardware for isoelectric focusing from Biometra.

are 0.1–10-mm thick and up to 10 cm in length. Mini-Focus can be used in conjunction with Biometra's Minigel apparatus for 2-D electrophoresis.

International Biotechnologies, Inc. has a programmable **polarity switching system** for the separation of large DNA molecules (*Reader Service No. 105*). The Fiji HV system comes in two models: the $2,450 (US) HV600 and the $2,950 (US) HV3000, which have voltage ranges of 0–700 V and 0–3,000 V, respectively. The two-component system consists of a switching device that can control up to four gels simultaneously, and a remote keypad. The system automatically ramps time intervals and can switch polarity at intervals as short as 0.001 seconds, says IBI. Operating parameters are entered using the menu-driven programming interface, which can store up to 99 switching protocols. IBI says the system has applications in megabase gene mapping, gene linkage studies, and in restriction fragment mapping of lambda, cosmid and yeast clones.

Chromatography columns

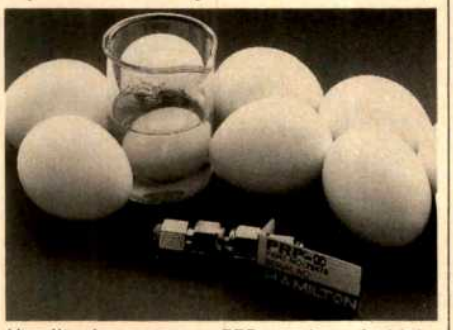
The process-scale purification of proteins, peptides or other biomolecules need not be a problem with the new range of **glass chromatography columns** from Pharmacia LKB (*Reader Service No. 106*). BioProcess Glass Columns have a 3-bar operating pressure and are available with diameters of 100 or 200 mm and lengths of 500, 750 or 950 mm. The single-screw adaptor can



Glass chromatography column for process-scale protein purification from Pharmacia.

be adjusted to give a wide range of bed heights and bed volumes and makes even contact with the gel bed. Designed for hygienic operation and maintenance, the column is made of borosilicate glass and electropolished steel, which Pharmacia says resists corrosion, reduces friction and hinders microbial attachment. Column packing is a simple procedure as the gel bed is clearly visible through the graduated glass tube. The column can be used for many separation techniques, including desalting/buffer exchange, ion exchange, affinity chromatography, hydrophobic interaction and gel filtration.

Hamilton Co. says its new polymeric **reversed phase HPLC column** can resolve high molecular weight proteins that differ by only a few amino acids (*Reader Service No. 107*). The $290 (US) non-porous PRP-∞ column can produce gradient separations of six proteins in 60 seconds,



Hamilton's non-porous PRP-∞ column for high-speed protein separations.

keeping peaks narrow for improved detectability and high resolution, says the company. The 30 × 4.1-mm column is packed with a polystyrene-divinyl benzene material that is stable up to pressures of 5,000 p.s.i. The stability of the column from pH 1 to 13 allows samples to be analysed at basic pH (8–13), which Hamilton says improves detectability and can provide confirmation of protein purity.

The GS Series of Asahipak GPC columns from Advanced Separation Technologies, Inc. have a packing material based on **hard gel polymers** high in hydroxyl content, and can be used for HPLC of both hydrophilic and hydrophobic substances (*Reader Service No. 108*). Using the Asahipak GPC columns, dual mode chromatography can be performed, which effectively combines both gel permeation chromatography and adsorption chromatography in the same column. The matrix can withstand high pressures and high flow rates, and is stable up to pH 14, says the company. A wide range of organic solvents can be used with GPC columns, says ASTEC, without the bed shrinkage associated with conventional packing materials. Asahipak columns can be used for the separation of proteins, enzymes, nucleic acids, peptides, vitamins, polysaccharides and oligosaccharides, sugars, and drugs and metabolites in body fluids. The columns are available in analytical and preparative sizes and prices range from $800 to $6,150 (US).

A new line of **multi-dimensional polymeric columns** that combine the power of ion exchange, reversed-phase separation and ion pairing into a single column are available from Dionex (*Reader Service No. 109*). Users can elute both inorganic and organic analytes in the same run, attaining a selectivity that Dionex says cannot be achieved using single mode separation analysis. The first two columns from the OmniPac line are the OmniPac PAX-500 and PAX-100. These 4 × 250-mm columns have identical ion-exchange properties, but the PAX-100 has lower reversed-phase characteristics. The ion exchange sites are very hydrophilic and have a high affinity for hydroxide anions. Dionex says that due to the excellent bed stability of Omnipac columns, they are 100 per cent solvent-compatible and stable over the entire pH range of 0–14. Dionex will introduce columns with cation exchange and reversed-phase properties in the autumn.

Centrifugal separation

A microprocessor-controlled **high-speed microcentrifuge** that automatically compensates for unbalanced tubes and converts r.p.m. to *g*-values is available from Camlab Ltd (*Reader Service No. 110*).

The model 157 has an operating speed spanning 100 to 30,000 *g*. The centrifuge recognizes the code of the rotor in use and ensures that the *g*-value setting is correct and does not exceed the maximum permissible value. Up to 99 programs can be entered and stored, and a large 80-character LCD provides the user with details of the preprogrammed values. The model 157 is available with and without refrigeration, for £3,790 (UK) and £2,490 (UK), respectively. Camlab offers 30 types of rotor for use with the centrifuge, each costing between £100–300 (UK).

Alfa-Laval Sharples Ltd says its Centritech Cell is a new type of separation system for **concentrating mammalian and other cell types** that are sensitive to shear forces (*Reader Service No. 111*). The company says the system has been used successfully to concentrate feline leukaemia cells from 2.58 to 36.7 million cells per millilitre, with 99.9 per cent cell viability after separation. The completely enclosed system offers no risk of contamination of cells or media and no risk to the user from harmful products. The company

Alfa-Laval's Centritech Cell, separation for shear-sensitive cells.

says the machine provides gentle acceleration and exerts the minimum centrifugal force required to effect separation. The system offers continuous separation, and at preprogrammed intervals the cell concentrate and clarified media are automatically discharged from a disposable bladder.

Beckman Instruments, Inc. has a new class of **ultracentrifuge** featuring a drive mechanism that is so stress-tolerant that rotor loads can be balanced by eye (*Reader Service No. 112*). The $31,500-36,500 (US) L and $37,000-42,000 (US) LX Optima Series operate without CFCs or liquid coolants over a temperature range of 0–40 °C. The XL models, which have menu-driven operation, provide automatic rotor logging, run data recording, and storage of ten run programs with up to five speed changes per run. The XL's calculation function can simplify and speed up run conversions by performing many of the calculations necessary for CsCl and sucrose gradients. The XL's Efficient Sedimentation Program automatically adjusts speed and time to provide efficient run times without gradient precipitation, and provides run simulations that forecast the condition of the gradient and separation components. The L Series has many of the control features of the XL Series, including the ability to store programs.

Modular HPLC systems

Perkin-Elmer has introduced a gradient LC system for high-resolution **peptide mapping** (*Reader Service No. 113*). The modular system comprises an injector, printer/plotter, Vydac C18 analytical column, scavenger column for cleaning out contaminants in the mobile phase, LC-135 dual channel detector, and model 250 Binary Pumps. The $19,600 (US) system is configured specifically for high-sensitivity peptide mapping, which Perkin-Elmer says has a 5–10 pmol sensitivity and provides the flow rate precision necessary to reproduce peptide maps exactly.

Hitachi announces its new line of **components and systems for HPLC** that can be configured together to form a stand-alone system with the flexibility of a modular design (*Reader Service No. 114*). Hitachi offers isocratic and gradient pumping systems made of both stainless steel and inert components and costing between $4,495–10,680 (US). The $4,495 (US) model 4200 variable wavelength detector offers low noise and a maximum sensitivity of 0.001 AUFS says Hitachi: both UV and UV/visible versions are available. A $8,995 (US) Diode Array Detector can be used in conjunction with a PC-based software package for contour plotting, chromatogram overlays and 3-D data display. Alternatively, the system can be used with an integrator which offers complete systems control from one keyboard for single or dual channel operations. The integrator can store up to 600 chromatograms and has data acquisition and data processing capabilities.

Milton Roy LDC Analytical says its 4000 series low-pressure **gradient HPLC systems** offer both high performance and built-in flexibility (*Reader Service No. 115*). The $14,486 (US) model 4300 system includes a single pump that provides either binary or ternary gradients, a spectroMonitor UV/visible detector, Rheodyne injection valve and C18 column. The $16,854 (US) model 4400 system includes the above components but features a programmable multi-wavelength detector instead of the spectroMonitor, which the company says has a sensitivity of 0.0005 AUFS. The addition of a PC and LCadvantage software provides the capability for complete systems control and the capacity for data storage.

Autosamplers

High sample handling capacity and programmable pre-column derivatization are features of the Gynkotek HPLC **autosampler** from Roth Scientific Company Ltd (*Reader Service No. 116*). The Gina 160 can handle up to 160 samples; the capacity can be increased further by replacing the vials in one of the two sample trays during the run. Injection volumes can be selected from one of two ranges: 1–250 µl or 0.1–25 µl, but vials must contain a minimum volume of 5 µl. Operating

The programmable Gina 160 high-capacity autosampler from Roth Scientific.

parameters for start position, running time, repeat injections and column temperature can be programmed for each sample and are entered via a keyboard and displayed on an LCD. The sample needle is flushed with eluent after each injection to minimize carryover. The autosampler, costing less than £7,000 (UK), can be programmed for pre-column derivatization, where up to two reagents can be added, mixed, and injected into the column after a pre-selected reaction time has elapsed.

Designed for **automated sample transfer**, the new autosampler from Severn Analytical Ltd is compatible with any HPLC system or integrator (*Reader Service No. 117*). The £3,500 (UK) Model 728 uses positive sampling displacement to provide viscosity-independent sample transfer, eliminating the need for accessory gases, says the company. The autosampler can hold up to 64 sample vials and can make up to three injections per vial, rinsing the system after each injection. Sample vials are loaded into the control module before subsequent transfer to a fixed-loop injection valve and injection into the the HPLC column. A selection of loop sizes are available, including a variable loop version that allows the user to program a different sample injection volume for each sample. The Model 728 can be used to control integrators and other external components, or can itself be controlled by a PC. □

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

Nature Classified

LONDON Julie Skeet, 4 Little Essex St, London WC2R 3LF. Telephone: 01 872 0102. Fax: 01 240 2408.
NEW YORK – Marianne Ettisch, 65 Bleecker Street, New York, NY 10012. Telephone: (212) 477 9625. Fax: (212) 505 1364.
PARIS Clare Newell, BP No 804, 75828 Paris, Cedex 17. Tel: (1) 40 53 03 39.
MUNICH – Sabine Fürst, Herzog-Wilhelm Strasse 35, 8000 München 2. Telephone: (089) 26 50 32. Fax: (089) 26 93 24.
TOKYO – Phillip Hamill, Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162. Telephone: (03) 267 8751. Fax: (03) 267 8746.

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Department of Scientific and Industrial Research

BIO TECH

DSIR

IMMUNOLOGIST/ BIOCHEMIST

Biotechnology Division

The Department of Scientific and Industrial Research is the largest scientific research organisation in New Zealand. Three divisions (Biotechnology, Grasslands and Plant Physiology) are based in Palmerston North. The Immunology Group of Biotechnology Division, has an opening for an Immunologist/Biochemist. The appointee is required to extend an existing programme in monoclonal antibody production.

The successful candidate will have proven expertise in the fields of animal cell physiology/biochemistry. Specific skills in hybridoma technology, in vitro cell culture techniques, animal cell metabolism and protein biochemistry would be an advantage.

Palmerston North (population 70,000), is located in a pleasant rural area, about half an hours drive from the coast. The city caters for a wide range of recreational activities and has excellent educational facilities.

The position is for a term of two years. Salary will be commensurate with qualifications and experience.

Please apply in writing, enclosing a curriculum vitae which should include the names of two referees and a facsimile number by Friday 8 September, 1989, and post or facsimile to:

The Personnel Officer
DSIR, Private Bag
Palmerston North
NEW ZEALAND
Fax no. 0064-63-61130.

(W6449)A

PAKISTAN

ACADEMIC APPOINTMENTS FOR THE GHULAM ISHAQ KHAN (G.I.K.) INSTITUTE OF ENGINEERING SCIENCES AND TECHNOLOGY, TOPI, NORTH WESTERN FRONTIER PROVINCE, PAKISTAN

SOPREST (Society for the Promotion of Engineering Sciences & Technology in Pakistan) is a registered Society which is approved as a Charitable Institution by the Government of Pakistan.

Mr. Ghulam Ishaq Khan, President of the Islamic Republic of Pakistan, is one of the 12 Founding Members of the Society and also its President and Chief Executive Officer for life. Other Founding Members include eminent industrialists, scientists and engineers of the Country.

The objective of SOPREST is to establish centres of excellence in Pakistan in the *Private Sector* in the fields of modern engineering and technological education.

The G.I.K. Institute is the first centre of excellence proposed to be established by SOPREST in TOPI near the Scenic TARBELA DAM in the NWF Province of Pakistan. About 220 acres of land has already been donated by the N.W.F.P. Government for the Project site and detailed designing work is in progress.

The Institute will, over a period, provide educational and research facilities to at least 400 students every year ranging from the undergraduate levels to Ph.D. in the following disciplines:

1. Metallurgy and Materials Science
2. Mechanical Engineering
3. Computer Science
4. Electronic Engineering
5. Applied Physics and Mathematics

Vacancies under each of these disciplines in the following cadres would need to be filled in the near future by well qualified, internationally reputed and experienced candidates:

— **RECTOR OF THE INSTITUTE**
— **A DEAN for each of the Five Faculties**
— **PROFESSORS, ASSISTANT PROFESSORS AND RESEARCH ASSOCIATES**

) Salary and terms negotiable

Please apply with full curriculum vitae to:

Mr. H. U. Beg
Executive Director, SOPREST,
4/A, Street No. 41, F-7/1,
Islamabad, PAKISTAN.

(W6444)A

Yeast Molecular Biologist

The Brewing Research Foundation has an international reputation as a centre of excellence for research & development work in malting, brewing, fermentation and beer processing. It is situated in pleasant rural surroundings near Redhill in Surrey.

We now wish to appoint a high calibre molecular biologist to work in our Fermentation Section on the application of modern molecular genetic techniques to the fermentation process. This will include the development of methods for the differentiation of strains of brewing yeasts, the use of genetic techniques for the investigation of flavour production by yeast and the development of novel transformation systems for brewing yeasts. The post would suit those with qualifications in biochemistry, microbiology, molecular biology or related subjects. The successful candidate will have post graduate training in molecular biology and ideally post doctoral experience. Laboratory experience with yeast, though not essential, would be an advantage.

Conditions of service are good and the initial salary will reflect qualifications and experience. If you are interested in this employment opportunity, please write, enclosing a curriculum vitae, and the names of two referees to, **The Administrator, The Brewing Research Foundation, Lyttel Hall, Nutfield, Redhill, Surrey, RH1 4HY,** *quoting reference FE02* (1446)A

POSTDOCTORAL RESEARCH FELLOW

AFRC PLANT MOLECULAR BIOLOGY INITIATIVE
DEPARTMENT OF BIOLOGY

Applications are invited for a postdoctoral research fellowship, tenable from 1 October 1989 (or as soon as practicable thereafter) to investigate the origin an molecular organisation of "unselfish" B-chromosomes in Allium schoenoprasum.

Applicants should have, or expect to obtain in the near future, a PhD in a biological subject. Experience of molecular biological techniques would be an advantage.

The appointment will be up to 3 years on Research and Analogous Grade 1A £10,458-£16,665 per annum.

Informal enquiries to Dr S M Bougourd, Department of Biology, tel (0904) 432807.

Further details may be obtained from the Personnel Office to whom two copies of applications with full curriculum vitae and naming two referees, should be sent as soon as possible to the Personnel Office, University of York, Heslington, York YO1 5DD: please quote reference number 6/6271. (1468)A

UNIVERSITY OF YORK

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
P.O. BOX 30772, NAIROBI, KENYA

Applications are invited from suitably qualified candidates for the following positions tenable at the ICIPE's Mbita Point Field Station, ICIPE's major Field Station, on the shores of Lake Victoria, 500 kilometres west of Nairobi. The Field Station has an International School and an Institutional Clinic.

POSTDOCTORAL RESEARCH FELLOW
CROP PESTS RESEARCH PROGRAMME
REF: SCI/89/1

Qualifications and Experience: The successful candidate will have a Ph.D. degree in agricultural entomology. Preference will be given to a candidate having experience in the field of host plant resistance to insects and insect behaviour.

Duties and Responsibilities: The selected candidate will be expected to conduct research on the host-plant resistance in maize, sorghum, cowpea and rice to stem borers and other insect pests in collaboration with other scientists under the overall supervision of the Programme Leader of Crop Pests Research Programme.

POSTDOCTORAL RESEARCH FELLOW
CROP PESTS RESEARCH PROGRAMME
REF: SCI/89/3

A vacancy exists for the position of Postdoctoral Research Fellow (ICIPE/DANIDA Collaborative Project on the Cassava Green Spider mite).

Qualifications and Experience: The successful candidate will have a Ph.D. degree in entomology or a related discipline. He should also have carried out research in insect pathology especially in the areas of mycology and virology. Ability to work for long hours in the field and a strong drive for research in challenging and unchartered waters will be an advantage.

Duties and Responsibilities: The successful applicant will be expected to carry out research on fungal and viral pathogens of the cassava green mite *Mononychellus tanajoa*, and determine the potential of selected pathogens for field control of the mite. He will work under the supervision of a Senior Research Scientist, under the overall guidance of the Research Leader, Biological Control Sub-Programme.

POSTDOCTORAL RESEARCH FELLOW
(AGRONOMIST)
CROP PESTS RESEARCH PROGRAMME
REF: SCI/89/4

Qualifications and Experience: Candidates should have a Ph.D. degree in any of the Agricultural Sciences, preferably in Agronomy with a strong background in plant breeding. Experience in on-farm experiments and interactions with both resource-poor farmers and national programmes will be definitely an advantage.

Duties and Responsibilities: The successful candidate will be required to work alongside a multidisciplinary team of scientists under the Crop Pests Research Programme, investigating the impact of some of the cropping patterns as components of pest management.

POSTDOCTORAL RESEARCH FELLOW
(GENETICS)
CROP PESTS RESEARCH PROGRAMME
REF: SCI/89/5

Qualifications and Experience: The successful candidate will have a Ph.D. in entomology or related subject with research experience in insect genetics.

Duties and Responsibilities: The selected candidate will be expected to conduct research on development of genetic methods for cereal stem borers control particularly through induction of hybrid sterility and elucidate the basic genetics of *Chilo partellus*.

SENIOR RESEARCH SCIENTIST
CROP PESTS RESEARCH PROGRAMME
REF: SCI/89/12

Qualifications and Experience: The successful candidate will have a Ph.D. in biology, a strong background in biometrics and several publications demonstrating this background. Candidates with a degree in statistics and having some biological experience will also be considered.

Duties and Responsibilities: The successful candidate will be required to consult closely with scientists in the Crop Pests Research Programme and the Biomathematics Research Unit; in addition, he will be expected to develop his own research area, preferably in the population dynamics of lepidopterous pests of maize, sorghum or cowpea. Data analysis will require familiarization with micro and mini-computers.

RESEARCH ASSOCIATE
CROP PESTS RESEARCH PROGRAMME
REF: SCI/89/13

Qualifications and Experience: The successful candidate will have a B.Sc. degree in agricultural sciences with training and considerable experience of breeding crops, particularly maize or sorghum. Possession of at least the M.Sc. degree will be an advantage.

Duties and Responsibilities: The successful candidate will be required to carry out maize or sorghum breeding in collaboration with the entomologists under the Leader of the Crop Pests Research Programme, with a view to incorporating pest resistance factors into improved varieties with acceptable agronomic characters. The selected candidate will be seconded to the ICIPE for an initial period of two years.

The renumeration package attached to these positions is negotiable and includes attractive benefits.

Applications giving detailed information on education, professional qualifications, experience, detailed and updated curriculum vitae, key reprints, marital status, age, present employment (including salary), names and addresses of four referees (one being personal), and photocopies of relevant certificates (which will not be returned), should be addressed to the undersigned not later than 2nd October 1989.

The Manager for Administration and Information
The International Centre of Insect
Physiology and Ecology (ICIPE)
P.O. Box 30772
NAIROBI

(W6445)A

MIAMI UNIVERSITY

CHAIR, DEPARTMENT OF BOTANY

Position: Miami University invites applications and nominations for the position of Chair of the Department of Botany. Candidates must have an excellent record of accomplishment in research within the discipline, a demonstrated interest and ability in effective teaching, and the capability of providing the administrative leadership to continue and extend the department's established strengths in research, teaching, and service. The successful candidate will begin a five-year renewable term July 1, 1990.

Department: The Department of Botany, with seventeen faculty, offers the Baccalaureate, Masters, and Ph.D. degrees. The department occupies the upper floor of a new biological sciences building. Special research facilities include an electron microscopy center, the Willard Sherman Turrell Herbarium, research greenhouses, a plant growth chamber facility, a 160-hectare ecology research center, and computer and microcomputer facilities. Departmental research interests include: ecology, molecular biology, morphology, mycology, phycology, plant anatomy, plant physiology, systematics and tissue culture. Major interdisciplinary projects have been developed with faculty of Chemistry, Microbiology and Zoology Departments.

Application: Review of applications will commence on October 13, 1989. Application should include a curriculum vitae, and descriptions of: administrative experience, teaching experience and current research, including copies of representative publications. All above material and five letters of reference should be sent to: **Dr. John Skillings, Chair, Botany Chair Search Committee, College of Arts and Science, 270 Upham Hall, Miami University, Oxford, Ohio 45056. (513) 529-5818.** *Equal Opportunity in Employment and Education.* (NW4111)A

John Radcliffe Hospital, Oxford OX3 9DU (0865) 64711

CARDIAC SURGEON'S ASSISTANT

SALARY: Negotiable up to £16,390 per annum.

This is a newly created post in Oxford and the first of its kind in the United Kingdom.

The Surgeon's Assistant will be part of the Cardiac Surgical Team. We expect the successful candidate to have advance knowledge of anatomy and physiology, and pharmacology with a nursing background or possibly be someone with proven expertise in dissection with a University background.

One of the varied duties of the Surgeon's Assistant will be the surgical removal of the long saphenous vein for coronary artery bypass surgery.

A four month training programme will take place at the Cleveland Clinic, Cleveland, Ohio, U.S.A. In order to maximise on this training we expect the successful candidate to commit themselves to the role, and in time, to be involved in the development of Surgeon's Assistant training in Oxford. Shortlisted candidates will be required prior to interview to discuss the role fully with the Cardiac Surgeon and spend some time in our general theatres.

If you feel that you have the ability to respond to this challenging opportunity then please contact Mike Flemming, Unit Personnel Manager, John Radcliffe Hospital on Oxford (0865) 817520.

Application form and post details available from the Personnel Department, Level 3, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Closing date – Friday 15th September 1989.

(1463)A

Oxfordshire Health Authority

NATIONAL INSTITUTE ON AGING
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH AND HUMAN SERVICES

NMR Spectroscopists

(2 Positions Available)

The Laboratory of Cellular and Molecular Biology at the Gerontology Research Center, National Institute on Aging, National Institutes of Health, PHS, DHHS is accepting applications for two available positions.

One position is for studies on the effect of aging on metabolic changes that occur during human exercise, as well as in vivo studies on animals using a Bruker Biospec 1.9 T/31 cm Spectrometer. The successful applicant for this position will bear primary responsibility for in vivo NMR operations in the laboratory, will collaborate on the ongoing programs, and will initiate other studies.

The second position is for collaborative studies on the mechanisms of RNA synthesis at the active site of RNA polymerase, being investigated using a Varian XL-200 NMR spectrometer, an IBM-200-D EPR spectrometer and a variety of other techniques.

Salary dependent on qualifications.

Other NMR instruments in the area are available for use in these studies.

A letter of application with a curriculum vitae and bibliography should be submitted to:

Dr. Gunther L. Eichhorn
Chief, Laboratory of Cellular and Molecular Biology
National Institute on Aging, Intramural Research Program
4940 Eastern Avenue
Baltimore, Maryland 21224

NATIONAL INSTITUTES OF HEALTH

Further information can be obtained by contacting Dr. Eichhorn at (301) 550-1807.

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW4116)A

Chief, Data Systems Operations Branch

The Science Computing and Research Support Division of the Space Telescope Science Institute invites applications for the position of Chief, Data Systems Operations Branch (DSOB). The appointment is expected to be at the Associate or Full Astronomer level, corresponding with Associate or Full Professor at a university.

The individual we are seeking is expected to conduct a continuing vigorous research program in HST-related atronomy. Access to the HST data archive will be a powerful research tool the incumbent may use in the Archival Research era.

The DSOB Chief will be responsible for overseeing all activities of the Branch. These activities include operating the Data Management Facility (DMF), an optical disk based system for archiving and distributing Hubble Space Telescope data. Making the HST archive easily accessible and more useful is one of the most important responsibilities. Scientific oversight of the development and eventual operations of the next generation archive (DADS) will be undertaken by the DSOB. The incumbent will also provide technical management of the Photolab.

Candidates should possess a Ph.D. or equivalent degree in astronomy or physics with five or more years of research experience with a significant publication record. Experience in handling and managing large quantities of digital scientific data is required, along with strong verbal and written communication skills. Some experience in managing data technicians and/or research assistants is preferred.

The STScI is strongly committed to equal opportunity. Women and minorities are strongly urged to apply.

SPACE TELESCOPE SCIENCE INSTITUTE

Please submit resume, bibliography and research plans and the names of four references by October 31, 1989 to Robert Teleky, Personnel Officer, Space Telescope Science Institute, 3700 San Martin Drive, Baltimore, Md. 21218. (NW4127)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY
POSTDOCTORAL MOLECULAR BIOLOGIST (1)
RESEARCH ASSISTANT (1)

The Human Molecular Genetics Group invites applications for two positions on a new project funded by the Human Genome Mapping Project of the MRC. The aim of the project is to construct a detailed map of chromosome 9. This will involve identifying loci manifesting DNA length polymorphisms and examining recombination between such loci among flow-sorted single sperm using the polymerase chain reaction on single molecules.

The successful applicant for a three-year position as a postdoctoral molecular biologist should have a Ph.D. or be submitting a Ph.D. thesis. Salary on University age-related scale. For further details please contact Professor M. A. Ferguson-Smith, Dr. N. A. Affara or Dr. M. A. R. Yuille on (0223) 333713.

The successful applicant for a three year position as a research assistant should have experience in basic recombinant DNA techniques, including the plating and screening of libraries. Qualifications: HNC/ Degree. Salary dependent on age and experience.

For either position, please make application in writing with c.v. and names and addresses of two referees to **The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP.** Please quote 11650.

The University follows an equal opportunities policy. (1443)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

TECHNICIAN

Required to work on the molecular and cell biology of wound healing. The project will involve culture of keratinocytes for experiments on cytokine expression. Some animal work will also be involved. Candidates are sought with a background in tissue culture methodology. Experience in preparation and manipulation of plasmid DNA would be an additional advantage.
Salary: £8,682-£10,289 per annum depending on qualifications and experience.
Applications to **Dr I McKay, Department of Dermatology, The London Hospital, Whitechapel, London E1.**
Further enquiries to Dr McKay on 01-377 7749 or 01-377 7724. (1449)A

Bayerische Julius-Maximilians-Universität Würzburg

An der Medizinischen Fakultät der Universität Würzburg ist die Stelle

einer Universitätsprofessorin/eines Universitätsprofessors der BesGr. C 4 für Physiologische Chemie I (Lehrstuhl)

(Nachfolge Professor Dr. E. Helmreich)

zu besetzen.

Zu den Aufgaben der/des zuküftigen Stelleninhaberin/Stellen-inhabers gehört die Vertretung des Fachs in in Forschung und Lehre im neu gegründeten interdisziplinären Biozentrum der Universität Würzburg.

Einstellungsvoraussetzungen sind abgeschlossenes Hochschulstudium, pädagogische Eignung, Promotion und Habilitation.

Die Bewerberin/der Bewerber darf das 52. Lebensjahr im Zeitpunkt der Ernennung noch nicht vollendet haben.

Bewerbungen sind mit den üblichen Unterlagen (Lebenslauf, Zeugnisse, Urkunden, Schriftenverzeichnis) bis zum **10. Oktober 1989** zu richten an

**den Dekan der Medizinischen Fakultät,
Herrn Prof. Dr. K Kochsiek, Josef-Schneider-Str. 2,
D–8700 Würzburg, F.R.G.** (W6441)A

MASSEY UNIVERSITY

DEAN OF THE FACULTY OF VETERINARY SCIENCE

The Council of the University invites applications for the appointment of Dean of Veterinary Science.

The position provides the opportunity to play a major role in the further development of teaching and research in the Faculty and more generally within the University. The Veterinary School has now been producing B.V.Sc graduates for 25 years and impending retirements in the Faculty provide a unique opportunity to shape future development. The appointee should hold a veterinary degree registrable in New Zealand and have appropriate postgraduate qualifications and experience. The Dean will be expected to maintain and promote the high international standing of the B.V.Sc degree. Massey University has the only Faculty of Veterinary Science in New Zealand and liaison with other major veterinary schools, with the veterinary profession and with consumers of veterinary services will be important aspects of the position.

This appointment, which would carry with it professional title, status and salary would be for an initial period of five years with the possibility of re-appointment thereafter.

Salary payable is in the range of $NZ76,000 to $NZ95,000.

Closing date 30 November 1989.

UGC POSTDOCTORAL RESEARCH FELLOWSHIP
Pentose Sugar Utilisation of Pachysolen tannophilus

A position is available in the Microbiology and Genetics Department under the leadership of Dr. R J Thornton, to conduct genetic and physiological studies of pentose sugar utilisation by the yeast Pachysolen tannophilus.

Catabolite repression and fermentation parameters affecting the rate and yield of ethanol production from pentose sugars will be investigated. Emphasis will be placed on a molecular biology approach to these problems. Existing hexose negative strains will be utilised to aid these investigations and in a hybridisation programme to develop strains with superior ability to convert pentose sugars to ethanol in the presence of hexose sugars. Experience with the use of molecular biology, biochemical, physiological and microbiological techniques in relation to yeast is desirable.

The annual emolument payable for Postdoctoral Research Fellowships is currently $NZ35,000 p.a. and the Fellowship is tenable for a maximum period of two years.

Closing date is 30 November 1989.

Further details of the above positions together with Conditions of Appointment are obtainable from Mrs V B Bretherton, Personnel Section, to whom applications, including a full curriculum vitae and the names of three referees should be sent before the closing date specified.

B.R.H. Monks
Registrar

We are Equal Opportunity Employer

**Palmerston North
New Zealand
Telephone (064) 63 69-099**

(W6439)A

TENURE-TRACK ASTRONOMERS

The Space Telescope Science Institute (ST ScI) has several openings for tenure-track astronomers. Excellence in research is the primary qualification for these positions. The level of the appointments — assistant, associate, or full astronomer — will be determined by the experience and accomplishments of the successful applicants. Tenure-track astronomers at ST ScI spend about 50% of their time in personal research and the remainder in support of the science mission of the Hubble Space Telescope. There is some latitude within this framework to tailor the functional component of the positions to the interests and expertise of the successful applicants.

ST ScI supports the research of its scientific staff through visitor, postdoctoral, and graduate-student programs. Several workshops and symposia are held each year at the Institute. Internal funds are available for travel to scientific meetings and observing sites, and computing and other equipment. Members of the staff may obtain additional support from any of the regular NASA grant programs. The salaries and benefits of tenure-track astronomers are commensurate with those at AURA member universities (without the need to apply for summer support).

Candidates for these positions must have a Ph.D. or equivalent degree in astronomy or physics and some post-doctoral experience. ST ScI is committed to equal opportunity; women and minorities are strongly urged to apply. Candidates should send a curriculum vitae and bibliography, a brief statement of research and functional interests, and the names of four references to the Personnel Manager.

SPACE TELESCOPE SCIENCE INSTITUTE

Space Telescope Science Institute
3700 San Martin Drive
Baltimore, MD 21218
Equal Opportunity Employer

Applications will be accepted until 31, December 1989. (NW4128)A

National Institutes of Health
National Institute of Dental Research

Chief, Laboratory of Developmental Biology and Anomalies

The Intramural Research Program of the National Institute of Dental Research invites nominations and applications for the position of Chief, Laboratory of Developmental Biology and Anomalies.

The Chief, Laboratory of Developmental Biology and Anomalies will be responsible for planning, directing, and conducting research programs which use biological, genetic and molecular approaches to study normal development and its aberrations. Appointee will have latitude in the recruitment of other senior scientists, post-doctoral fellows, and technicians.

Applicants should have a Doctoral degree or equivalent with accomplishments in the general area of developmental biology and with demonstrated ability for administrative leadership. Position is available immediately. This is a Civil Service position with a salary range of $57,158 to $74,303 per annum. Physicians and dentists may be eligible for Physician's Comparability Allowance of up to $20,000 per year. Alternatively, appointment in the Commissioned Corps of the U.S. Public Health Service may be appropriate. U.S. Citizenship is required.

Applicants must submit curriculum vitae, brief statement of research directions, and the names of three references. Civil Service applicants also must submit a SF-171. Documents must be submitted no later than September 30, 1989 to:

Ms. Judith T. Dulovich
Personnel Management Specialist
National Institute of Dental Research
National Institutes of Health
Building 31, Room 2C23
9000 Rockville Pike
Bethesda, MD 20892

NATIONAL INSTITUTES OF HEALTH

NIH is an Affirmative Action/Equal Opportunity Employer.

(NW4110)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE
PUBLIC HEALTH LABORATORY SERVICE AND SOUTH GLAMORGAN HEALTH AUTHORITY

CHAIR OF MEDICAL MICROBIOLOGY

Following a review of medical microbiological services in South Glamorgan commissioned by the Secretary of State for Wales, the College of Medicine invites applications for the Clinical Chair of Medical Microbiology, the holder of which will also be Director of the Cardiff Laboratory of Public Health Laboratory Service and honorary Consultant Manager of Medical Microbiological Services for South Glamorgan Health Authority.

The salary will be on the scale for Clinical Professors (currently up to a maximum of £38,340 per annum), augmented for the special duties of the post.

Further particulars may be obtained from the **Registrar and Secretary (Personnel Office), University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN (Tel. No. 0222/755944 Ext. 2296)** to whom applications including a curriculum vitae with the names and addresses of three referees should be sent by 7th October, 1989. (1448)A

MAX-PLANCK INSTITUTE FEUR SYSTEMPHYSIOLOGIE
DORTMUND WEST GERMANY

Post doctoral position

A position is available for ongoing work related to the molecular cloning of membrane transport proteins. Experience in current molecular biological techniques essential.

Applicants should posses a Ph.D. or equivalent and have a record of scientific accomplishment. Position is, in the first instance, for two years.

Please send curriculum vitae and names of three references to:

Alison I. Morrison Ph.D
Max-Planck Institut feur Systemphysiologie
Rheinlanddamm 201
4600 Dortmund 1
West Germany

(W6438)A

THE UNIVERSITY OF BIRMINGHAM
SCHOOL OF BIOLOGICAL SCIENCES

LECTURESHIP IN MICROBIAL MOLECULAR GENETICS

A position is available for an active research scientist in the Microbial Molecular Genetics and Cell Biology Group of the School of Biological Sciences (the former Departments of Genetics, Microbiology, Plant Biology and Zoology). The lectureship is associated with the appointment of Professor Nigel Brown to the Chair of Molecular Genetics and Microbiology.

Preference will be given to applicants with experience in the molecular biology of bacterial or other microbial systems. The overriding criterion will be academic excellence. The successful applicant will be expected to develop an independent research programme and to participate in teaching. Opportunities will exist to collaborate with other members of the School. The appointment will be on the Lecturer Scale, £10458-20469 plus superannuation.

For an informal discussion of the position please contact Professor N.L. Brown (Tel 021-414-6556, Fax 021-414-5925). For further particulars and an application form telephone 021-414-6483 (24 hour answerphone) quoting reference BS/2004. Applications (3 copies) should be sent to the **Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham, B15 2TT** by 20th October.

AN EQUAL OPPORTUNITIES EMPLOYER (1388)A

THE UNIVERSITY OF SHEFFIELD
Department of Molecular Biology & Biotechnology

Research Assistant and Post-doctoral Positions

Two positions are available immediately to study the Interaction of Human Immunoglobulin E with Class-specific Fc-receptors, continuing work described in Nature **331**, 180–183, 1988, and Nature **338**, 649–650, 1989. The appointments are funded by a collaborative award from the MRC and Industry for an initial period of three years and will be in the IA range, with salary according to age and experience.

A PhD in biochemistry, molecular biology, or immunology together with gene cloning, expression in heterologous expression systems, site-directed mutagenesis and PCR are preferred; but candidates with relevant technical experience are also encouraged to apply. Registration for a higher degree may be possible.

Applications with the names of two referees should be addressed to: **Dr B A Helm, University of Sheffield, Sheffield ST10 2TN** from whom further information can be obtained. Closing date for applications 29 September 1989. Reference R934/G.

An Equal Opportunity Employer (1456)A

British Bio-technology is a research and development company in health sciences. Utilising both molecular biology and medicinal chemistry techniques, the company aims to bring innovative pharmaceutical products to the human health care market in the 1990's and beyond.

A Career at the Leading Edge of Health Science

British Bio-technology Limited

CELL BIOLOGIST

We are currently seeking a post doctoral Cell Biologist who will strengthen our research into new products with inflammation and wound healing properties. Appropriate experience with growth factors or cytokines and preferably receptors would be of special interest. (Job Ref: 176)

The opportunities for advancement are excellent in this friendly and expanding company and the benefits include -

- **First class pension scheme**
- **Individual health care scheme**
- **Share option scheme**
- **Social Club**
- **Free life assurance**
- **25 days ' holiday p.a.**

Write with details of your career to date to:

Rod Cook,
Head of Personnel,
British Bio-technology Limited,
Watlington Road, Cowley, Oxford OX4 5LY
Telephone: (0865) 748747

(1467)A

University of Leicester — John Innes Institute

PROTEIN CHEMIST/ENZYMOLOGIST

Applications are invited for a postdoctoral Research Associate to join an interdisciplinary group studying the enzymology of antibiotic biosynthesis in *Streptomyces*. This is part of an AFRC Linked Research Group between the Department of Chemistry, University of Leicester (Professor T J Simpson) and the Department of Genetics, John Innes Institute, Norwich (Professor D A Hopwood). The appointee will be based in Leicester but will visit Norwich on a regular basis for research planning and discussions. The project will involve isolation and purification of biosynthetic enzymes from genetically engineered strains for structural studies and for mechanistic studies of the reactions catalysed by the enzymes. This post is available immediately for a 3-year period.

Salary on the RA IA scale £10,458-£16,665 a year, according to qualifications and experience. Applications including a C.V. and the names of two referees should be sent to **Professor T J Simpson, Department of Chemistry, University of Leicester, Leicester LE1 7RH (Tel: 0533-522107)** from whom further information may be obtained. (1458)A

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE
(University of London)
DEPARTMENT OF HAEMATOLOGY

POST-DOCTORAL RESEARCH ASSISTANT

Required to work with Dr R.G. Wickremasinghe and Professor A.V. Hoffbrand on the mechanisms of growth factor signal transduction in normal and leukaemic human haemopoietic cells. Experience in one or more of the following areas would be an advantage: cell culture, protein chemistry, second messenger systems, molecular biology. Informal inquiries may be addressed to Dr Wickremasinghe on Tel: (01) 794 0500 (Ext: 3478). The post is funded for three years by the Kay Kendall Leukaemia Fund.

Salary on Range 1A: £10,458-£16,665 plus £1,650 London Allowance.

Job description available from the **School Office, R.F.H.S.M., Rowland Hill Street, London NW3 2PF** (Tel: 01-794 0500 Ext: 4262) to which applications (**TWO** copies of curriculum vitae with names and addresses of **two** referees) should be sent by **15 September 1989.** Please quote Ref: **HAE/RA/22.** (1454)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL
(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG
DEPARTMENT OF MEDICINE

An EXPERIENCED POSTDOCTORAL RESEARCH ASSISTANT

is invited to join a project which aims to define T cell subsets which mediate beneficial and harmful immunity to RS virus. Work will include establishment and testing of T cell lines and clones, measurement of cytokine release from T cells in response to recombinant viral antigens and use of recombinant vaccinia viruses. The applicant will be expected to show drive and initiative; initial salary up to £15,372 pa + £1650 pa London Allowance depending on qualifications and experience.

For further details please 'phone Dr. Peter Openshaw (01) 725-6302. Informal visits are welcome.

Applications in form of a full C.V. with names, addresses and 'phone numbers of 2 referees should be sent to **Personnel Department** at above address. Please quote Ref: PO/PRA. (1470)A

POSTDOCTORAL RESEARCH ASSOCIATE

required for research in mammalian

TESTICULAR FUNCTION

in particular, on the role of gonadotropins and growth factors in spermatogenesis. Experience in modern techniques of reproductive endocrinology, receptor biology or molecular biology expected. Funds, technical assistance and animal models including non-human primates available. Knowledge of German not required.

The Institute covers research on the control of testicular function, male infertility, male contraception and human in vitro fertilisation.

Applications to:
Prof. Dr. E. Nieschlag,
Institute of Reproductive Medicine of the University,
Steinfurter Str. 107,
D-4400 Münster/F.R. Germany. (W6440)A

UNIVERSITY OF
essex

Department of Biology

Molecular Biology of Photosynthesis

Applications are invited for 4 AFRC-funded positions, three postdoctoral Senior Research Officers and one graduate Research Officer, to join an expanding team studying the development of the photosynthetic apparatus. An integrated approach will be taken to investigate environmental effects on nuclear and chloroplast proteins and genes, aiming to identify the protein factors involved in transcriptional and post-transcriptional regulation. Applicants should have some experience of either molecular biology or protein biochemistry and an interest in chloroplast development. Informal enquiries to Dr. C.A. Raines (0206 873310) or Professor N.R. Baker (0206 873319).

Salaries for the postdoctoral positions will be in the range £10,458 - £13,527 per annum on Grade IA and for the graduate position in the range £9,816 - £10,458 per annum on Grade IB.

Applications (three copies), including a curriculum vitae and the names and addresses of two referees, should reach the Registrar (R/957/N), University of Essex, Wivenhoe Park, Colchester, CO4 3SQ by 21 September 1989. Further particulars of this post may be obtained by telephoning Colchester (0206) 872462 (24 hours). (1433)A

INSTITUTE OF PLANT SCIENCE RESEARCH
CAMBRIDGE LABORATORY

Four Molecular Biologists

Four 3-year appointments are available, funded by the Agricultural Genetics Company, to join a group working on the isolation of agronomically important genes from cereals. The work is being performed in collaboration with another team generating an RFLP map to wheat and barley. The project will entail the use of chromosome walking techniques to extend the RFLP maps of 2 wheat chromosome groups carrying some of the most important cereal traits, with the aim of cloning and characterising the genes responsible. The work will include an assessment of the use of YAC technology in the context of the barley and wheat genomes.

One appointment will be at the Higher Scientific Officer level, for which candidates should have experience in recombinant DNA techniques. Candidates should have a good honours degree, and PhD and/or relevant experience.

The other three appointments will be at the Scientific Officer level, for which experience in recombinant DNA techniques would be an advantage. Candidates should have an honours degree in a relevant biological subject, or HNC with relevant post qualification experience.

The salary scale at HSO level is £10,678 p.a.-£14,909 p.a. and SO level £9,131 p.a.-£12,223 p.a. subject to experience.

Initially the successful candidates will be located at the Cambridge Laboratory, but on April 1st 1990 they will be re-located, together with other staff, to new laboratories on the John Innes site at Norwich.

Applications with full curriculum vitae and the names of three referees to **Mrs L Cliff, Institute of Plant Science Research (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB**, quoting reference **CR/367** by 22nd September 1989.

The Institute is an Equal Opportunities Employer. (1453)A

Hillingdon Health Authority
Department of Research in Plastic Surgery
Regional Plastic, Maxillofacial & Oral Surgery Centre
Mount Vernon Hospital
RESEARCH FELLOW

Salary in the range of: £15,899-£19,827 plus £757 London Weighting Allowance, according to qualifications and experience.

The Department of Research in Plastic Surgery is expanding its interests in the effects of pressure on tissues and the prevention of tissue damage using appropriate support systems. A new research post has been established to lead this project.

The successful applicant will have a degree in biological science and experience in clinical measurement. The post which is funded by R.A.F.T. — The Restoration of Appearance and Function Trust is for five years in the first instance.

Applications including full C.V. and names and addresses of two referees should be sent to **Professor J T Scales O.B.E., Department of Research in Plastic Surgery, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN.**

Closing date: Tuesday 31st October 1989.

Working Towards Equal Opportunities (1442)A

University of Bristol
DEPARTMENT OF PATHOLOGY
Lecturer in Veterinary Pathology

Applications are invited from Veterinary Graduates for a post of Lecturer in Veterinary Pathology under the New Academic Appointments Scheme.

Salary on the Lecturer scale £10,458–£20,469 per annum.

The Department of Pathology is responsible for teaching Medical, Dental and Honours Science students and is involved in a wide range of research projects, including immunology, microbiology and oncology.

The post will be based primarily at the University of Bristol Veterinary School, Langford. Applications should have research experience using molecular biological and/or immunological techniques applied to the field of animal health and disease. Duties will include teaching Veterinary Science students as well as involvement in the provision of a diagnostic pathology service. Experience of diagnostic pathology would be advantageous but is not essential.

Applications (in duplicate), giving the names and addresses of three referees should be sent by 22nd September to the **Registrar, University of Bristol, Senate House, Bristol BS8 1TH** from whom further particulars may be obtained. Please quote reference PR/Med/7. Enquiries may be made to Professor Silver, Department of Pathology, Tel: 0272 303030 ext. 4445.

AN EQUAL OPPORTUNITY EMPLOYER. (1436)A

UNITED MEDICAL AND DENTAL SCHOOLS
of Guy's and St Thomas's Hospitals
Guy's Campus — close London Bridge

RESEARCH ASSISTANT required to work in the Allergy Unit at Guy's Campus. The work will involve the study of Biochemistry of Cytokines. Experience in Protein or Carbohydrate Biochemistry would be preferred. Possession of a PhD would be helpful but not essential.

Post would be for two years. Salary in the range £8,675-£12,381 pa plus £1,650 pa London Weighting allowance.

For further information/informal visit please contact Professor T. Lee on 01-955 5000 ext 4571. To apply, please send two copies of your CV together with the names and addresses of two referees to **The Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH**, quoting ref no. **G/MED/393.**

(1438)A

Medical Research Council
Brain Metabolism Unit — Edinburgh
SCIENTIFIC OFFICER

Applications are invited for the post of Scientific Officer. The person appointed will work on the mechanism of action of stress neuropeptides under the direct supervision of Dr Ferenc Antoni. Experience in cell culturing, cell biology and/or the biochemistry of transmembrane signalling would be an advantage. The person appointed must hold at least a BSc or HNC or equivalent.

The appointment will be made on the Scientific Officer scale, salary in the range £8,574-£10,994.

Further information may be obtained from **Mrs J Dalitz, Administrator, MRC Brain Metabolism Unit, University Department of Pharmacology, 1 George Square, Edinburgh EH8 9JZ.** Applications, including a curriculum vitae and the names of three professional referees, should be sent to the Administrator. The closing date is 19 September 1989.

MRC

The MRC is an equal opportunity employer. (1254)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
P.O. BOX 30772, NAIROBI, KENYA

RESEARCH SCIENTIST/SENIOR RESEARCH SCIENTIST (POPULATION BIOLOGY/MODELLING) CROP PESTS RESEARCH PROGRAMME REF: SCI/89/14

Qualifications and Experience: The successful candidate will have a Ph.D. degree in insect science with at least three years of postdoctoral research experience as evidenced by referred publications in the field of Population Biology and Modelling with relevance to insects.

Duties and Responsibilities: The selected candidate will be expected to conduct and supervise research on various aspects of Population Biology of selected insect pests of crops and develop population models and forecasting systems for these pests. The research will be carried out in collaboration with a team of scientists under the overall supervision of the Leader of the Crop Pests Research Programme.

SCIENTIST-IN-RESIDENCE (POPULATION BIOLOGY/MODELLING) CROP PESTS RESEARCH PROGRAMME REF: SCI/89/15

Qualifications and Experience: The candidates must have a Ph.D. followed by at least 10 years of research experience supported by referred publications in the field of Population Biology and Modelling of insects.

Duties and Responsibilities: The candidate will be expected to conduct and supervise research on various aspects of Population Biology of selected insect pests and develop population models and forecasting systems for these pests. The research will be carried out in collaboration with a team of scientists under the overall supervision of the Leader of the Crop Pests Research Programme. This position is tenable for 9-12 months.

RESEARCH SCIENTIST/SENIOR RESEARCH SCIENTIST (BIOLOGICAL CONTROL) REF: SCI/89/16

Qualifications and Experience: Candidates must have a Ph.D. degree in entomology and at least three years of post-doctoral experience, as evidenced by referred publications, in the field of biological control of insect pests using parasitoids.

Duties and Responsibilities: The selected candidate will be expected to work in the area of insect parasitoids, and will conduct and supervise research on parasitoids of crop pests for utilisation in integrated Pest Management research programmes. The research will be carried out in collaboration with a team of scientists under the overall supervision of the Programme Leader, Crop Pests Research Programme.

The following positions are tenable at the ICIPE Headquarters in Nairobi, Kenya.

POSTDOCTORAL RESEARCH FELLOW CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT REF: SCI/89/6

Qualifications and Experience: Applicants must have a Ph.D. degree in Organic Chemistry with proficiency in chromatographic separation (HPLC, GC, etc) and spectral identification of natural products (MS, NMR). Experience in semiochemical work and in synthesis will be additional assets.

Duties and Responsibilities: The successful candidate will join a team of scientists working on the chemical ecology of lepidopteran crop pests of maize, sorghum and cowpea and of mammalian disease vectors such as tsetse flies and sand flies. He will be required to undertake the identification of behaviour-controlling chemicals of a selected insect, working in close collaboration with entomologists, and contribute to related activities of the Unit.

POSTDOCTORAL RESEARCH FELLOW TSETSE RESEARCH PROGRAMME REF: SCI/89/2

Qualifications and Experience: The successful candidate will have a Ph.D. degree in insect science with experience in the field of insect behaviour or ecology.

Duties and Responsibilities: The successful candidate will join a team of scientists working on the epidemiology of African trypanosomiasis. He will carry out a study on the effectiveness of tsetse control operation in localities with more than species of tsetse.

POSTDOCTORAL RESEARCH FELLOW CHEMISTRY AND BIOCHEMISTRY RESEARCH UNIT REF: SCI/89/10

Qualifications and Experience: Applications for this position should have recently received their Ph.Ds in Biochemistry or Molecular Biology. Previous experience in modern methods of separation and characterization of biologically active proteins would be valuable, as well as established experience in DNA/RNA characterization.

Duties and Responsibilities: The successful candidate will join a small group of scientists working on two main areas: (i) biochemical basis of diapause in the stem borer, *Busseola fusca*, and parasite-vector interactions in African trypanosomiasis and (ii) identification of tick-derived antigens with potential for inducing resistance in host animals.

PESTNET RESIDENT SCIENTIST (KENYA) REF: SCI/89/17

Qualifications and Experience: Candidates must have a Ph.D. degree in entomology or related sciences. Post-doctoral research experience is desirable, but thorough knowledge of English and working knowledge of Swahili are essential.

Duties and Responsibilities: The successful applicant will work interactively with the National Programme scientists to generate basic information on the relevant aspects of the biology, behaviour and population dynamics of target pests of priority food crops in the country. In a joint effort with the National Programme scientists, the successful candidate will conduct field tests on specific IPM components developed by the ICIPE and other institutions, for effectiveness, appropriateness and adaptability in different agroecological zones in the country. The appointee will also be required to play an active role in the integration of promising IPM components into low-cost IPM technologies for the management of the target pests and provide effective contact for close collaboration between the ICIPE and the National Programme.

POSTDOCTORAL RESEARCH FELLOW, SOCIAL SCIENCE INTERFACE RESEARCH UNIT REF: SCI/89/19

The position is based at the ICIPE Headquarters, Nairobi, with possibility of relocation and/or field assignments to sites within Kenya.

Qualifications and Experience: Candidates should have a Ph.D. degree in sociology and athropology.

Duties and Responsibilities: The selected candidate will work closely with biologists researching on malaria and leishmaniasis problems in various parts of Kenya. He will be responsible for the development of socio-economic baseline data on the two human diseases, conducting research on popular perceptions and indigenous knowledge systems, and assessing the impact of the ICIPE's Integrated Pest Management (IPM) packages.

The fellowship for postdoctoral research fellows is tenable for a period of two years.

The renumeration package attached to these positions is negotiable, and included attractive benefits.

Applications giving detailed information on general education, professional qualifications, experience, marital status, age, present employment, names and addresses of four referees and photocopies of relevant certificates should be addressed to the undersigned not later than 2nd October 1989.

The Manager for Administration and Information
The International Centre of Insect
Physiology and Ecology (ICIPE)
P.O. Box 30772
NAIROBI.

W(6446)A

THE INTERNATIONAL CENTRE OF INSECT PHYSIOLOGY AND ECOLOGY
P.O. BOX 30772, NAIROBI KENYA

HEAD OF TRAINING
REF: ADM/89/30

Applications are invited from suitably qualified candidates for the position of HEAD OF TRAINING at the International Centre of Insect Physiology and Ecology (ICIPE). The ICIPE conducts research into insect crop pests and the vectors of human and animal tropical diseases using a mission oriented and multidisciplinary approach. The Centre has a major commitment to training and educational programmes in insect science for both practitioners and scientists from tropical developing countries, with an emphasis on Africa. The ICIPE is also the executing agency for the African Regional Postgraduate Programme in Insect Science (ARPPIS). ARPPIS is a collaborative postgraduate training programme between the ICIPE and 14 participating universities in Africa. It offers Ph.D. training at the ICIPE in a 3-year course which includes teaching and research. The graduate educational activities of the ICIPE, which are already well recognized (with formal links to the Association of African Universities and the Network of Deans and Directors of Graduate Studies of Southern and Eastern African Universities) are being further strengthened and developed.

Qualifications and Experience: The candidate should have research and teaching experience in the biological sciences; and have been at least a senior lecturer for a minimum of 5 years, preferably in an African University. Experience in the administration and management of training, particularly post-graduate studies, is essential, and first hand knowledge of African Universities and of networking will be advantageous.

Responsibilities: The selected candidate will assist the ICIPE in the planning, implementation and coordination of all training activities at the ICIPE (in-service training, short courses, research associates, postdoctoral research fellowships and graduate education), and will work closely with colleagues in the ICIPE, and in national research systems, including universities.

The responsibilities include the direction of the study programmes for the up to 40 graduate students resident at the ICIPE at any one time. As part of the responsibility for training the appointee will be the secretary to the Academic Board of ARPPIS and the Chairman of the ARPPIS Board of Studies; and will work closely with ARPPIS participating universities to establish sub-regional centres for ARPPIS M.Sc. degrees in insect science. The selected candidate will be expected to play a major role in planning the envisaged strengthening of graduate training at the ICIPE.

Applications and resume giving detailed information on general education, professional qualifications, a track record of professional experience, marital status, age, present terms of employment, names and addresses of four referees, and photocopies of relevant certificates should be addressed to the undersigned to reach him not later than 2nd October 1989.

The Manager for Administration and Information
The International Centre of Insect
Physiology and Ecology
P.O. Box 30772
Nairobi

(W6447)A

LEADING LATIN AMERICAN MARINE SCIENCE CENTER OF A SOUTHERN BRAZILIAN UNIVERSITY HAS OPENINGS FOR RESEARCH AND POSTGRADUATE TEACHING POSITIONS IN:

CHEMICAL OCEANOGRAPHY
PHYSICAL OCEANOGRAPHY
BIOSTATISTICS
MARINE MICROBIOLOGY
ZOOPLANKTON BIOLOGY
PHYTOPLANKTON BIOLOGY
PRIMARY PRODUCTION
COASTAL PLANT ECOLOGY
MALACOLOGY
MARINE ORNITHOLOGY
MARICULTURE

CANDIDATES WITH DOCTORAL DEGREE SHOULD CONTACT:
Dr. José A. Levy, Curso de Pos-Graduação em Oceanografia Universidade do Rio Grande, Caixa Postal, 474, 96200 Rio Grande — RS Brazil. (W6448)A

AFRC INSTITUTE FOR GRASSLAND AND ANIMAL PRODUCTION
WELSH PLANT BREEDING STATION ABERYSTWYTH
CEREAL BREEDER
(Temporary Appointment)

Applications are invited for a Higher Scientific Officer in the Crop Improvement Department to carry out breeding of triticale. The programme, funded by Semundo Ltd, is aimed at producing new triticale varieties suitable as an alternative cereal requiring minimum inputs for growing in the UK and worldwide.

The successful applicant will also be expected to carry out a personal research programme aimed at improving the potential of triticale for production and use.

Qualifications
First or upper second class honours degree in an agricultural or biological science with at least 2 years' relevant post-graduate experience. Experience in plant breeding is desirable.

Terms and Conditions
Salary scale: £10,678-£14,909.

Non-contributory superannuation scheme.

Annual leave: 25 days plus 10.5 Public & Privilege holidays per annum.

Written requests for further particulars and application forms should be made to the Secretary, AFRC Institute for Grassland and Animal Production, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, SY23 3EB. (Quote Ref. 89/26).

Closing date: 20th September 1989.

The AFRC is an Equal Opportunities Employer. (1429)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY & RESEARCH
DIVISION OF BIOLOGICS
EUROPEAN COLLECTION OF ANIMAL CELL CULTURES
BASIC GRADE MICROBIOLOGIST — CELL CULTURE DEVELOPMENT

The function of the Collection is to store, characterise and distribute cell lines, contract cell culture and to act as an Internationally recognised Patent Depository. Cell culture development is a rapidly expanding area involving R & D relating to large scale cell culture.

Duties will include tissue culture, cell storage techniques, the production of working cell banks and seed cultures and the running of pilot scale bioreactor systems.

The post is suitable for a graduate with an appropriate degree and ideally relevant experience of aseptic techniques.

Basic Grade Microbiologist Salary Scale from £7378-£11073 (currently under review). Appointment on the scale according to qualifications and experience.

National Health Service terms and conditions will apply.

The post is funded for two years in the first instance.

The post may be discussed informally with Mr D R Cameron, telephone 0980 610391, ext 594.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilts SP4 0JG. Telephone (0980) 610391, ext 240, to whom completed forms should be returned by 15th September 1989 quoting Post No. 0556.

(1437)A

UNIVERSITY OF GLASGOW
DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY
POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a post-doctoral research assistantship supported by the Arthritis and Rheumatism Council to study the locomotion and adhesion of lymphocyte maturation subsets and their relation to chronic inlammation. The project will involve assays of lymphocyte locomotion and clustering with accessory cells, and of the locomotor properties of different subsets defined with monoclonal antibodies. It will require a background in immunology or cell biology. Funding is available for 3 years on the Grade 1A scale (£10,458-11,736) plus superannuation.
Applications with C.V. and names and addresses of two referees to **Professor P. C. Wilkinson, Department of Bacteriology and Immunology, Western Infirmary, Glasgow, G11 6NT**, by the end of September. (1461)A

ASSISTANT/ASSOCIATE PROFESSORS IN MICROBIOLOGY/ IMMUNOLOGY

Several tenure-track faculty positions are available to talented investigators who can establish independent, high quality research programs in molecular aspects of virology and immunology. Excellent new facilities and attractive start-up funds available. Submit curriculum vitae, statement of research interest and list of three references to: **Dr. Katherine Knight, Chairperson, Department of Microbiology, Loyola University of Chicago, Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153.** *Loyola University of Chicago is an Equal Opportunity/Affirmative Action Employer.* (NW4113)A

INSTITUTE OF PLANT SCIENCE RESEARCH, CAMBRIDGE LABORATORY

POSTDOCTORAL and TECHNICAL ASSISTANT POSTS

The Molecular Genetics Department of the Institute of Plant Science Research (Cambridge Laboratory), formerly the Plant Breeding Institute, is initiating a major research programme in the genetics and molecular biology of *Arabidopsis thaliana*. This programme will include an EEC funded centre for physical mapping of the *Arabidopsis* genome. Research posts are available in the following projects, funded as part of the AFRC initiative in *Arabidopsis* molecular biology.

1. Development of an efficient transposon-tagging system in *Arabidopsis thaliana*. One post at postdoctoral (HSO), and one at technical assistant (SO) level. PG111/576.

2. Construction of an overlapping genomic library of *Arabidopsis* DNA in yeast artificial chromosome (YAC) vectors. Two posts, both at technical assistant (SO) level. PG111/575.

3. Molecular genetic analysis of the vernalization requirement in *Arabidopsis thaliana*. One post at postdoctoral (HSO), and one at technical assistant (SO) level. PG111/574.

4. Identification and isolation of genes involved in determining the flowering time of *Arabidopsis thaliana* in response to daylength. One post at postdoctoral (HSO) and one at technical assistant (SO) level. PG111/577.

5. Genetic and molecular analysis of gibberellin biosynthesis and signal-transduction in *Arabidopsis thaliana*. One post at postdoctoral (HSO) and one at technical assistant (SO) level. PG111/578.

The programme is being led by Drs George Coupland (1, 2 and 4), Caroline Dean (1, 2 and 3) and Nicholas Harberd (1, 2 and 5). The department is moving to new premises on the site of the John Innes Institute in Norwich in March 1990. The laboratory is well equipped, and has all the facilities necessary for research in plant molecular biology. In addition, there is considerable in-house experience in techniques such as plant transformation, DNA sequencing etc. The Norwich site promises to be an exciting venue for plant science research since it will be occupied by the John Innes Institute, the Sainsbury Laboratory, and the Cambridge Laboratory, thus facilitating interactions between plant biologists in different disciplines.

All posts are available for a period of 3 years. Applicants should have an interest in plant biology (although we welcome applications from those wishing to move into this area), and some experience in molecular biology, biochemistry, or genetics. Applicants for HSO should have an honours degree in a relevant discipline, together with at least 2 years' relevant post-graduate research or relevant experience, and applicants for SO should have an honours degree.

The salary scale at HSO level is £10,678-£14,909 p.a. and SO level £9,131-£12,223 p.a. starting point subject to qualifications and experience.

Applications should be made to **Mrs. L. Cliff, Institute of Plant Science Research, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB**, identifying the project(s) in which you are interested, and including a curriculum vitae and the names of three referees by 22nd September, 1989.

The Institute is an Equal Opportunities Employer. (1445)A

MIT

It's where you want to be

Postdoctoral Scientists

MIT Whitaker College of Health Sciences and Technology

Recently, a means has been found here to directly observe point mutational spectra in the DNA of cell and tissue preparations. We have created several new postdoctoral positions for persons with a background in molecular biology or analytical chemistry who wish to join us in our initial studies using the new technology. Opportunity exists for study of mutational mechanisms in human cells or bacteria, genetic epidemiology in humans or studies of mutations in oncogenes arising in carcinogen-treated animals.

All positions are fully funded with a salary of up to $25,000 per annum. Opportunity exists for submitting RO grant applications for exceptional individuals. Please submit CV, bibliography and references to: **William G. Thilly, Professor of Applied Biology, Whitaker College of Health Sciences and Technology, MIT, Bldg. E18-666, 400 Main Street, Cambridge, MA 02139.**

MIT is an Equal Opportunity/Affirmative Action Employer
MIT is a non-smoking environment (NW4132)A

Massachusetts Institute of Technology

SENIOR NEUROBIOLOGIST/NEUROSCIENTIST

National Institute of Mental Health Intramural Research Program

The NIMH is accepting applications for a senior neurobiologist/neuroscientist with interests in studying basic brain mechanisms of behavior. Applicants must hold a doctoral degree or equivalent and be interested in joining an established research program involving behavioral neurophysiology, neuroanatomy, neuropsychology, developmental neurobiology, neuroethology and other disciplines. The laboratory facilities are located in a rural setting approximately 30 miles west of the main NIH Bethesda campus near the town of Poolesville, Maryland. Researchers in the fields of neurophysiology, neuroanatomy, experimental psychology (including physiological psychology), developmental neurobiology, and related fields in the brain and behavioral sciences are especially encouraged to apply. Excellent facilities exist for primate research. The successful candidate should have broad experience and established credentials in the neurosciences. Duties will include scientific leadership and administration of a laboratory, as well as conduct of an internationally-recognized research program. Salary will be commensurate with other NIMH researchers having similar responsibilities, approximately $57,158 to $75,500 plus full Federal benefits. Send C.V., bibliography, a statement of future research directions, and the names of three references to:

Steven M. Paul, M.D.
Acting Director, Intramural Research Program
National Institute of Mental Health
Building 10, Room 4N-224
9000 Rockville Pike
Bethesda, Maryland 20892

NATIONAL INSTITUTES OF HEALTH

NIMH IS AN EQUAL OPPORTUNITY EMPLOYER (NW4089)A

BIOCHEMISTRY, BIOPHYSICS AND MOLECULAR BIOLOGY
UNIVERSITY OF IOWA
COLLEGE OF MEDICINE

The Department of Biochemistry of the University of Iowa seeks highly qualified applicants for a faculty position at the Assistant Professor (tenure-track) to Professor (tenured) level. Salary will be competitive and will depend on qualifications and experience. Support for equipping laboratories and initiating research programs will be excellent. Applicants with research in any area of biochemistry will be considered. Quality of research, not a specific research area, will be the primary criterion.

All applicants must have a relevant graduate degree (Ph.D., M.D. [or both] or equivalent foreign degree). Applicants for Assistant Professor rank should have at least two years of productive postdoctoral experience and the potential to initiate and maintain a rigorous research program. Applicants for Associate or Full Professor rank should have well-funded, on-going research programs and significant national stature in their research areas. In addition to establishing or continuing successful research programs, faculty participate in the department teaching programs. The training of graduate students and participation in appropriate graduate programs also is expected. Applications should include a complete Curriculum Vitae and a summary of future research plans. Applicants for a junior faculty position should ask three scientists familiar with their work to write letters of reference. Applicants for a senior position should include the names of at least three references. Applications, nomination and letters of reference should be sent to: **Alan G. Goodridge, Professor and Head, Department of Biochemistry, College of Medicine, University of Iowa, Iowa City, IA 52242.**

The University of Iowa is an Affirmative Action/Equal Opportunity Employer. (NW4120)A

UNIVERSITY COLLEGE LONDON
THE GALTON LABORATORY
POSTDOCTORAL RESEARCH ASSISTANT

This position is available from October 1989 working with Dr. S. Povey and Dr. J. Wolfe to develop a method to clone human genes from somatic cell hybrids containing only small regions of the human genome. Molecular biology experience is desirable, preferably with some experience in one or more of the areas of PCR amplification, DNA sequencing or cDNA cloning. The position is funded by an MRC Human Genome Mapping Project grant and is for three years; salary at an appropriate point according to age and experience on the research assistant 1A scale (£10458-£13527 plus London Allowance £1650).

Further information can be obtained from **Dr. Wolfe (01 387 7050 x5073/6), The Galton Laboratory, 4 Stephenson Way, London NW1 2HE**, to whom applications including a c.v. and the names of two academic referees should be sent by September 22nd 1989. *Equal Opportunities Employer.* (1462)A

University of Toronto
MRC Group in Periodontal Physiology
Post-doctoral/Research Associate Position

An exciting opportunity exists for a post-doctoral/Research Associate position to study the interaction between osteoblasts and osteoclasts in the regulation of bone resorption. The work will involve in vitro techniques of cell and organ culture, purification and analysis of conditioned media for the purpose of finding bone resorption stimulating factors and identification of osteoclast-specific genes activated during the resorptive phase of osteoclastic resorption.

The successful candidate should have completed her/his doctoral thesis, and have experience with HPLC and the handling and purification of peptides. Experience in tissue culture techniques and scanning electron microscopy would be an asset, although not essential.

Salary is at the Research Associate level, and will be approximately $30,000 per year. The position will be available Sept. 1st, 1989, will be for 2 to 3 years initially.

Applications should be sent, together with a full curriculum vitae and the names and addresses of three referees to: **Dr. Johan N.M. Heersche, MRC Group in Periodontal Physiology, Room 4384 Medical Sciences Building, University of Toronto, Toronto, Ontario M5S 1A8,** Telephone No.: (416) 978-6684, from whom additional information can be obtained. (NW4122)A

AFRC INSTITUTE FOR GRASSLAND AND ANIMAL PRODUCTION
WELSH PLANT BREEDING STATION ABERYSTWYTH
PLANT SCIENTISTS (Temporary Appointments)

Applications are invited for three posts funded by the AFRC Plant Molecular Biology Initiative. The projects are concerned with:

1. Sucrose-induced gene expression and protein targeting in leaves of graminaceous plants (Ref 89/28).

2. Genetic manipulation of tannin biosynthetic pathways in forage legumes (Ref 89/29).

3. Elucidation of genetic constraints upon low temperature growth in grasses and cereals (Ref 89/30).

The Higher Scientific Officer posts will be for a fixed term of 3 years in the first instance.

Qualifications: First or upper second class honours degree in Biochemistry, Botany, Cell Biology, Genetics or Molecular Biology with at least two years' post graduate experience. Familiarity with molecular biology or plant biochemistry techniques would be of particular advantage.

Terms & Conditions: Salary scale (under review): HSO — £10,026-£13,460. Non-contributory superannuation scheme. Annual leave: 25 days plus 10.5 Public & Privilege holidays per annum.

Written requests for further particulars and application forms should be made to the Secretary, AFRC Institute for Grassland and Animal Production, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB. (Quote Ref as above.)

Closing date: 18 September 1989.

The AFRC is an Equal Opportunity Employer. (1428)A

RESEARCH PSYCHIATRIST

Position available for conducting neuropsychiatric clinical research on eating/affective disorders and related molecular neurobiology studies. Previous training in neurology, psychiatry and neuropsychiatric research is essential. Candidates with additional experience in neurobiology laboratory research are preferred. Salary: $23,000-35,000, commensurate with experience. Send application and CV to **Philip W. Gold, M.D., Chief, Clinical Neuroendocrinology Branch, NIMH, Building 10, Room 3S-231, 9000 Rockville Pike, Bethesda, Maryland 20892.** *EOE M/F/H/V.* (NW4126)A

PUBLIC HEALTH LABORATORY SERVICE BOARD
PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH
RESEARCH ASSISTANT

A vacancy for an experienced research assistant has arisen within the Molecular Biology Group in the Division of Pathology. The person appointed will join an active research team working on the expression of human immunodeficiency virus genes in eukaryotic host environments. Applicants should have experience of genetic manipulation techniques; tissue culture experience, although not essential, will be advantageous.

This position is funded by the AIDs Directed Programme of the MRC and is for a fixed term of one year in the first instance. The post will be graded at either MLSO II, MPT III or BGM depending on qualifications, age and experience. The salary range is £7378 £11073 National Health Service terms and conditions will apply.

Further information about the position can be obtained from Dr A Akrigg; application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG. Telephone number 0980 610391. Please quote post no: 1127/29. (1431)A

MOLECULAR BIOLOGY

ASSISTANT PROFESSOR position is available in molecular biology for the study of the construction of chimeric genes and their expression. A competitive salary will be offered.

Applicants should submit their curriculum vitae and the names and addresses of two references to: **Dr. Constantin Bona, Microbiology Dept., Box 1124, The Mt. Sinai School of Medicine, One Gustave Levy Place, N.Y., N.Y. 10029.** (NW4114)A

POSTDOCTORAL POSITIONS IN RADIOBIOLOGY

Two post-doctoral positions are open in a well established laboratory studying low-level radiation effects. Candidates with experience in reproductive biology and/or tissue culture techniques are encouraged to apply. Please submit curriculum vitae to: **Prof. Roger W. Howell, Division of Radiation Research, MSB F-451, University of Medicine and Dentistry of New Jersey, 185 S. Orange Avenue, Newark, NJ 07103.** Telephone: (201) 456-5067, Fax: (201) 456-6474, The UMDNJ is an Affirmative Action/Equal Employment Opportunity Employer, M/F/H/V. (NW4129)A

UCL

PHOTOSYNTHESIS RESEARCH GROUP

POST-DOCTORAL RESEARCH ASSOCIATE

An SERC supported post-doctoral position is available for two years for work on the structure and function of photosystem 2. The research programme will involve work on the isolation of purified photosystem 2 preparations, and the application of advanced electron paramagnetic resonance spectroscopy to investigate the structure, and electron transfer mechanisms, in the oxygen evolving complex and the electron acceptor complex. A background in Biochemistry, Biophysics or Physical Chemistry would be appropriate. Training will be available for novel techniques. Salary £10,458-£12,381 plus London Allowance of £1,650.

Applications including c.v. and names and addresses of 2 referees should be sent to **Professor M.C.W. Evans, Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT. 01-380 7312.** From whom further details may be obtained. Equal Opportunities Employer. (1474)A

INSTITUTE OF ZOOLOGY
ZOOLOGICAL SOCIETY OF LONDON

LABORATORY TECHNICIAN

(ref: DHA/WLCM)

required to join research team investigating the physiological mechanism of social suppression of reproduction in marmoset monkeys, naked mole-rats and other exotic mammals. Minimum of three years laboratory experience essential, preferably in the field of reproductive endocrinology. Candidates should possess an HNC, BSc or equivalent qualification. Post available immediately and for three years in the first instance, at a starting salary within range £9,675-13,063 inclusive of London Weighting (under review).

For further details, please contact Dr Abbott (01-722 3333). Please apply in writing, indluding detailed CV, names of two referees and s.a.e. to the **Personnel Manager, London Zoo, Regent's Park, London NW1 4RY**, not later than 28th September, 1989. (1473)A

University of Oxford
Sir William Dunn School of Pathology

MOLECULAR VIROLOGY

5 year research position

Applications are invited for two postdoctoral fellows and one graduate research assistant to work on vaccinia virus molecular biology. The successful applicants will join an expanding research group in modern, well-equipped laboratories under the direction of Dr. G. L. Smith, to whom informal enquiries should be made (tel: 0865-275521). The positions are funded by an MRC programme grant with a starting date as soon as possible after October 1, 1989. Experience in the areas of recombinant DNA, protein chemistry and immunology would be advantageous but are not essential. Salary will be at the appropriate level on the academic related 1A (postdoctoral — £10,458-£16,665), or 1B (graduate — £9816-£14,169) scales.

Applications in writing, together with c.v. and names and addresses of two referees, should be sent to **The Administrator, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE.** Closing date for applications September 30, 1989.

An equal opportunity employer. (1441)A

RESEARCH ASSOCIATE

The Hematology/Oncology Section of the Department of Medicine has an immediate opening in cytokine research. The applicant must have a Ph.D. or M.S. degree with experience in the study of the effects of cytokines on hematopoiesis. Apply with curriculum vitae and three references to: **Dr. Ronald Hoffman, Chief, Hematology/Oncology, Indiana University School of Medicine, Clinical 379, 541 Clinical Drive, Indianapolis, IN 46202-5111.** ***An Equal Opportunity/Affirmative Action Employer*** (NW4131)A

Post Doctoral Research Associates

5 positions available this Fall for a multidisciplinary approach to study peptides and proteins for biological and clinical applications. Candidates should have experience in one of the following areas: 1) Peptide Synthesis 2) Immunology 3) Cell Biology 4) HIV 5) Blood Coagulation 6) Growth Factors.

Send curriculum vitae and names or letters of 2 references to: **Dr. James P. Tam, Rockefeller University, 1230 York Avenue, New York, NY 10021.** ***An Equal Opportunity Employer.*** **(NW4130)A**

POSTDOCTORAL POSITIONS PROTEIN CRYSTALLOGRAPHY

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

The Center for Macromolecular Crystallography has two postdoctoral positions available for a collaborative project with Schering Corporation on crystallographic studies of protein lymphokines (including interferons, interleukins and colony stimulating factors), their receptors, and the protein/receptor complexes. Crystals of two lymphokines (γ-interferon and GM-CSF) suitable for structural analysis are now in hand.

Fellowships are in the range of $26,000-$40,000, depending upon the experience and qualifications of the individuals. Initial appointments will be for a one-year period, with possible extension for additional years.

Send application letter with current CV and two references to: **Steven E. Ealick** or **Charles E. Bugg, Center for Macromolecular Crystallography, The University of Alabama at Birmingham, Box 79 THT, Birmingham, AL 35294.** (NW4100)A

UNIVERSITY OF LEICESTER

DEPARTMENT OF GENETICS

Two SERC-funded postdoctoral research posts are available in the laboratory of Dr. C. P. Kyriacou:

(1) on the molecular genetics of biological rhythms in *Drosophila*. An experienced molecular biologist is required who also has an interest in behaviour;

(2) on the genetic analysis of sexual behaviour in *Drosophila*. An interest in the evolution of sexual behaviour, plus some practical experience in genetics would be of value.

Both positions are available from 1 October 1989 for up to 3 years. Salary scale in the range £10,458-£16,665 a year depending on age and experience. Applicants should send a CV and the name and address of three referees to **Dr. C. P. Kyriacou, Department of Genetics, University of Leicester, Leicester LE1 7RH.** Informal enquiries can be made by telephone (0533-523430) to Dr. Kyriacou. (1459)A

THE UNIVERSITY OF BIRMINGHAM
SCHOOL OF MEDICAL SCIENCES

RESEARCH FELLOW 1A/II

Applications are invited for a one year postdoctoral fellowship, funded by The Leukaemia Research Fund, to join a group working on the growth and differentiation of human myeloid cells and the biology of myeloid leukaemias. The project will focus on intracellular events during myeloid cell diversification and in particular the identification of proteins which are phosphorylated and/or bind to DNA during this process.

Salary in the Grade 1A/II range — plus superannuation. Maximum starting salary £16,014.

Further details may be obtained from Drs. J.M. Lord and G. Brown (021 414 4074). Applications (three copies) with full curriculum vitae and the names and addresses of two referees to **Assistant Registrar, Medical School, Birmingham B15 2TJ** by 21st September, 1989. Please quote Ref. RF/IMM.

AN EQUAL OPPORTUNITIES EMPLOYER (1472)A

POSTDOCTORAL FELLOW

Position available at the National Institute on Alcohol Abuse and Alcoholism, for studies of basic mechanisms of receptor-effector coupling and neuropeptide actions in brain, and the effects of alcohol on these processes. Candidates should have a strong biochemical and/or pharmacological background, working knowledge of basic molecular biological techniques, and some postdoctoral experience. Salary is commensurate with training and experience. Only U.S. citizens or permanent residents are eligible for this position.

Send CV and three letters of recommendation to:

Dr. P. Hoffman
NIH
Building 10, Room 3C103
9000 Rockville Pike
Bethesda, MD 20892
Telephone (301) 443-5800

(NW4124)A

NIH is an Equal Opportunity Employer

POSTDOCTORAL POSITION IN THE MOLECULAR GENETICS AND BIOCHEMISTRY OF HERPES SIMPLEX VIRUS DNA REPLICATION

available immediately. Projects include: (i) molecular genetic analysis of functional domains of the HSV origin binding protein (UL9) and the components of the helicase/primase complex (UL5, UL8, and UL52) and (ii) elucidation of the role of viral enzymes such as alkaline nuclease in maturation and processing of replication and recombination intermediates. Experience in molecular biology and biochemistry is desirable.

Reply with curriculum vitae and names and addresses of three references: **Dr. Sandra K. Weller, Department of Microbiology, University of Connecticut School of Medicine, Farmington, CT 06032, (203) 679-2310**

AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER M/W/H.

(NW4123)A

ASSOCIATE or FULL PROFESSOR

The Medical College of Georgia invites applications from qualified individuals for a senior tenure-tract position in the Department of Cell and Molecular Biology. Preference will be given to candidates who have an active research program and who are using molecular approaches to study immunology or host parasite interactions. Applicants should have a proven ability to compete successfully for extramural funding. Academic responsibilities include both medical and graduate education. Administrative support for research and excellent facilities contribute to a strong research environment. The Medical College of Georgia, in Augusta, is located in a metropolitan area with a population of over 350,000, a strong cultural arts base, and a growing, diversified economy. Interested individuals should send by October 15th a brief letter of interest, a curriculum vitae, a copy of two recent publications, and the names and addresses of three references to **Dr. David F. Lapp, Chairman, Search Committee, Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-2100.**

An equal opportunity/affirmative action employer. (NW4119)A

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE

Applications are invited for postdoctoral positions in the following areas of

PLANT MOLECULAR BIOLOGY

(1) Development of expression vectors for monocotyledonous plant protoplasts based on a geminivirus replicon.
(2) Regulatory elements in geminivirus transcription.
(3) Geminiviruses as models for investigating plant DNA replication.
(4) Isolation and molecular characterisation of origins of DNA replication in *Arabidopsis*.

The post-doctoral assistants will join an active and enthusiastic team working in plant molecular biology, including the use of virus vectors and transgenic plants to study the regulation of gene expression. Excellent facilities for molecular biological and plant research are available. Salary will be on the RA 1A scale, in the range £12,738 to £14,031 p.a. including London Allowance. Applications, including a c.v., a list of publications, and the names of two referees should be made to **Prof. K.W. Buck, Department of Pure and Applied Biology, Imperial College of Science, Technology and Medicine, London SW7 2BB** as soon as possible and not later than 30 September, 1989. (1469)A

UNIVERSITY OF NEWCASTLE UPON TYNE

POSTDOCTORAL RESEARCH ASSOCIATE

Protein Folding

Applications are invited for a SERC funded postdoctoral research associate post which is available for 3 years to work on the pathway of folding of a large protein. The successful applicant will be setting up a new method involving rapid quenched flow and is expected to gain experience in making mutant proteins. Experience in handling proteins would be an advantage.

Starting salary will be £11,088 p.a. on the Grade 1A salary scale (£10,468-£16,665).

Please send applications, including curriculum vitae and the names of two referees to Professor R H Pain, Department of Biochemistry and Genetics, The University, Newcastle upon Tyne, NE2 4HH (Tel: 091 2327368).

(1450)A

The Department of Cell Biology and the Center for Reproductive Biology Research at Vanderbilt University

are seeking candidates for faculty positions at the Assistant Professor level. The appointees will be expected to develop an active and distinctive research program in one of the following areas of investigation: regulation of gene expression in reproductive tissues, cell or developmental biology of the male reproductive tract, and molecular biology of fertilization. Interested persons should send curriculum vitae and the names of three references to **M.-C. Orgebin-Crist, Ph.D., Director, Center for Reproductive Biology Research, Vanderbilt School of Medicine, Room D-2303 MCN, Nashville, Tennessee, 37232-2633.**

Vanderbilt is an equal opportunity/affirmative action employer. (NW4118)A

POST-DOCTORAL RESEARCH ASSOCIATE

to apply recombinant DNA technology to understand the role of a protein involved in microbial infection in humans. Competitive salary commensurate with experience. Send CV and at least three references to **Director, The Ohio State Biotechnology Center, The Ohio State University, Rightmire Hall, 1060 Carmack Road, Columbus, OH 43210.** *An equal opportunity/affirmative action employer.* (NW4125)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Biochemistry and Genetics

RESEARCH FELLOW

Applications are invited for a one year research appointment, at either postgraduate or postdoctoral level, funded by the University's Research Committee, to investigate structural aspects of cytochrome oxidase using techniques of protein chemistry.

Salary will be up to £12,381 per annum on either the Grade 1B or 1A scale according to the qualifications and experience of the successful candidate.

Applications with full curriculum vitae and the names and addresses of 3 referees, should be sent as soon as possible to Dr I C West, Department of Biochemistry and Genetics, The University, Catherine Cookson Building, Medical School, Newcastle upon Tyne NE2 4HH from whom further information may be obtained: Tel: 091 2226537. (1451)A

UNIVERSITY OF BRISTOL

Department of Oral Medicine, Surgery and Pathology

Research Technician Grade D

Applications are invited for the above post which will be of three year's duration in the first instance. The work will be connected with oral cancer. Previous experience with immunocytochemical techniques is essential and past work with tissue culture an advantage.

Salary will start at £8,904 rising to £9,446. Applicants should have H.N.C. or equivalent plus up to 5 years post-training experience.

Applications with c.v. should be submitted by 15th September 1989, to: **The Personnel Officer (EO), University of Bristol, Senate House, Tyndall Avenue, Bristol BS8 1TH,** quoting reference **A652**. Informal vists by potential applicants can be arranged by contacting: Mr D K Coles, Laboratory Superintendent, on (0272) 230050 Extension 4317.

An equal opportunities employer. (1378)A

U.S. DEPARTMENT OF AGRICULTURE
FOREST SERVICE
SOUTHERN FOREST EXPERIMENT STATION

PROJECT LEADER/SUPERVISORY RESEARCH ENTOMOLOGIST, RESEARCH FORESTER, RESEARCH ECOLOGIST, RESEARCH BIOLOGIST.

USDA-FS, South. For. Exp. Sta., is seeking a Research Entomologist, Research Forester, Research Ecologist, or Research Biologist to lead a team of researchers studying the population dynamics and interactions of the southern pine beetle, host trees, and stands to improve management of the pest and to develop practical means of control. Salary range is GM-13-15 ($41,121 to 57,158). Information concerning the position may be obtained from **Personnel Management, Rm. T-10210, USPSB, 701 Loyola Avenue, New Orleans, LA 70113 (504/589-3921).** The USDA is an Equal Opportunity Employer. (NW4092)A

INSTITUTE OF PLANT SCIENCE RESEARCH

CAMBRIDGE LABORATORY

Molecular Biologist

The Laboratory is seeking to appoint a Post-doctoral Fellow to carry out research on the transcriptional regulation of genes expressed in potato tubers. The work will involve gene isolation and characterization, expression vector construction, and plant transformation. The position would suit someone with a background in genetics, biochemistry or botany, with a particular interest in the application of genetic engineering to crop plants. Full training will be given.

The post is funded for two years. Initially the successful applicant will be based at the Cambridge Laboratory but in March 1990 will transfer to new laboratories at the John Innes Institute, Norwich.

The salary will be on the HSO scale £10,678-£14,909 with a non-contributory superannuation scheme.

Applications with curriculum vitae and the names and addresses of three referees to Mrs L Cliff, Institute of Plant Science Research (Cambridge Laboratory), Maris Lane, Trumpington, Cambridge CB2 2JB quoting reference PG111/568 by 22 September 1989.

The Institute is an Equal Opportunities Employer. (1464)A

UNIVERSITY OF NOTTINGHAM

Department of Biochemistry

POSTDOCTORAL RESEARCH FELLOWSHIPS AND RESEARCH ASSISTANTSHIP IN PROKARYOTIC MOLECULAR BIOLOGY TOWARDS THE ENGINEERING OF RNA POLYMERASE

Applications are invited for the posts of postdoctoral research fellow and research assistant supported by the MRC to join a large group concerned with fine structure analysis of the multimeric group of RNA polymerases. The project is multidisciplinary, employing recombinant, bacterial genetic, immunological and protein biochemical procedures. Applicants are expected to have a Ph.D./B.Sc. in a related area.

The posts, available for 3 years, are funded on the appropriate scale for research staff.

Applications, including a *curriculum vitae* and the names of two referees, should be sent to **Dr. Robert E. Glass, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH.** (Tel. 0602-709226). (1460)E

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London)

Ph.D. Student: Cytokines and Wound Healing in Skin

Candidates are sought for a project on the regulation of cytokine gene expression in human skin. The project can have a molecular or cell biological slant depending on candidates' background. Previous experience in cell transfection, gene expression or transcriptional regulation would be an advantage.

Salary: £3,970 per annum.

Applications to **Dr I McKay, Department of Dermatology, The London Hospital, Whitechapel, London E1.**

Further enquiries to Dr McKay on 01-377 7749 or 01-377 7724. (1455)A

RIVERSIDE HEALTH AUTHORITY

WESTMINSTER HOSPITAL

DATA MANAGER/ RESEARCH ASSISTANT

MEDICAL ONCOLOGY UNIT

Salary:– Approximately £13,000 (Inclusive of London Weighting).

Required for the Clinical Trials Office of the Medical Oncology Unit in this Hospital. The successful candidate will be responsible for the accurate retrieval, registration and processing of data, generated through the routine work and clinical trials conducted in this unit.

A background in science or nursing will be an advantage.

Priority will be given to applicants with computing skills and experience in biostatistics.

For further information contact Dr S. Retsas, Consultant Medical Oncologist, on 01-828-9811 ext 2546.

Application form and job description from the Unit Personnel Department, Queen Mary Nurses' Home, 20 Page Street, London, SW1P 2AP. Telephone 01-400 0296 (24 hour service).

Please quote reference:– DM/MO.

Closing Date:– Friday, 15 September 1989. (1465)A

JOHN INNES INSTITUTE

DEPARTMENT OF CELL BIOLOGY

CELL CYCLE GENES IN HIGHER PLANTS

Applications are invited for a higher Scientific Officer appointment, funded by the AFRC Plant Molecular Biology Programme, to study the cell division cycle in higher plants. The successful applicant will join a small team who are working to identify and characterise genes involved in mitosis. Candidates should have a research background in genetics and/or biochemistry. Although experience in molecular biolgy would be advantageous, training can be provided for otherwise suitable candidates. Informal enquiries are welcomed and should be made to Dr John Doonan or Dr Keith Roberts (Tel: (0603) 52571).

The appointment will be to the Higher Scientific Officer scale £10,678 to £14,335. Non contributory superannuation. The post will be available from October 1989 and for three years' duration.

Letters of application, together with full cv and names of two academic referees should be sent to the Personnel Officer, John Innes Institute, Colney Lane, Norwich NR4 7UH, quoting reference CB/399, to arrive not later than 29 September.

The John Innes Institute is associated with the AFRC Institute of Plant Science Research and is an Equal Opportunities Employer. (1466)A

FELLOWSHIPS

NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE

National Institutes of Health
Public Health Service

has immediate openings for:

MOLECULAR BIOLOGISTS, BIOCHEMISTS, COMPUTER PROGRAMMERS

for a

HUMAN GENOME SEQUENCING PROJECT

Fellowships available for molecular biologists/biochemists/computer programmers for a human genome sequencing project to sequence chromosomal regions including those implicated in fragile X syndrome and neurofibromatosis I. The large scale sequencing project involves automation of cloning, template preparation and sequencing techniques, implementation of robotics, and software development for sequence assembly and analysis.

Postdoctoral Fellowships Available:
Starting salary will be commensurate with experience.

For *IRTA Fellowship:* less than 3 years postdoctoral experience, US Citizenship required, $24,000 to $27,000

For *Fogarty Fellowship:* less than 3 years postdoctoral experience, Non-US Citizen, $20,000 to $23,000

For *Staff Fellowship:* over 3 years postdoctoral experience, US Citizenship required, $24,000 to $40.000

For *Senior Staff Fellowship:* over 3 years postdoctoral experience, US Citizenship required, $28,000 to $45,000

For further information and qualifications call:
Janet Claggett, NINDS Personnel Office
(301) 496-6334

Applicants should send their curriculim vitae and names of three references to:

J. Craig Venter, Ph.D.
NINDS/NIH
Park Building, Room 405
Bethesda, Maryland 20892

NIH IS AN EQUAL OPPORTUNITY EMPLOYER
(NW4115)E

HUMAN FRONTIER SCIENCE PROGRAM (HFSP)

The new international program starts now.

The Human Frontier Science Progrm (HFSP) is an international program, which was proposed by the Japanese Government at the Venice Economic Summit Meeting in 1987, and which has been discussed at the Toronto Economic Summit Meeting and at the meetings of eminent scientists and experts from the Economic Summit member countries and the Commission of the European Communities. The HFSP aims at promoting, through international cooperation, basic research focused on the elucidation of the sophisticated and complex mechanisms of living organisms. To the fullest possible extent the research results will be used for the benefit of all mankind. The HFSP is also intended to encourage international interdisciplinary research by researchers early in their careers who are expected to play an important role in originating and pursuing creative research.

Invitation for Applications

Research Grants/Fellowships/Workshops

Research Areas of the HFSP:

(A) Basic research for the elucidation of brain functions
1. Perception and Cognition
2. Movement and Behaviour
3. Memory and Learning
4. Language and Thinking

(B) Basic research for the elucidation of biological functions through molecular level approaches
1. Expression of Genetic Information
2. Morphogenesis
3. Molecular Recognition and Responses
4. Energy Conversion

Research grants: Grants for basic research carried out by international joint research teams represented by principal researchers from one of the eligible countries※.

Fellowships: Fellowships for researchers from the eligible countries who wish to do research in foreign countries, or for researchers outside the eligible countries who wish to do research in one of the eligible countries.

Workshops: Subsidies for international workshops organized by researchers from the eligible countries.

※ The eligible countries for fiscal year 1989 are Canada, France, F.R.G., Italy, Japan, U.K., and U.S.A.. Researchers in non-summit EC member countries may apply through the Commission of the EC on equal terms with the researchers in the eligible countries. (Details in the guidebook)

Prospective number of awards and duration of each activity for fiscal year 1989:

Research Grants	~ 20	3 years
Long-term Fellowships	~ 100	3 months - 2 years
Short-term Fellowships	~ 50	2 weeks - 3 months
Workshops	~ 10	----

Application Deadline: November 15, 1989
In fiscal year 1989, awardees will be determined by March 31, 1990, after the review procedures are completed by the organization to implement the HFSP.

Further information:
Until the organization is established in Strasbourg (France) this fall, the Task Force on the HFSP of the Japanese Government will undertake preparatory activities on behalf of the organization.

Guidebooks and Application Forms may be obtained upon request from the address given below. (Please indicate which program activity〔Research Grant/Fellowship/Workshop〕you are interested in.)

(W6443)G

The Task Force on the HFSP
c/o Science and Technology Agency, The Japanese Government
2-2-1, Kasumigaseki, Chiyoda-ku, Tokyo, 100, Japan.
FAX:(Japan 81)-3-581-3079, 3-581-5698, TEL:(Japan 81)-3-501-3490

HFSP

STA FELLOWSHIPS

Postdoctoral Fellowships in Japanese Government Laboratories

The Japanese Science and Technology Agency (STA) is offering postdoctoral fellowships to scientists and engineers of the countries listed below for periods of 6 months to 2 years to be held in any Japanese national laboratory (excluding university and university-affiliated laboratories). Over one hundred Japanese research laboratories covering almost all areas of science, engineering and medicine are participating in the scheme.

The fellowships are open to young PhD holders of under 35 (although older researchers will be considered) from universities, research councils, government research laboratories and industry. Any science or engineering discipline will be considered except military R & D. Applicants will be required to supply a letter of invitation from their Japanese host institution (the organizations listed in Table 1 provide help in contacting suitable host institutions).
Those in the final stages of a PhD may also apply.

There are no closing dates but candidates are encouraged to submit their applications as soon as possible. Fellowships for fiscal 1989 must be taken up by March 1990.

FELLOWSHIP AWARDS

Fellowships include round-trip air tickets (economy class) and the following tax-free allowances:

1) Living allowance: ¥270,000 (about US$2,000) a month
2) Family allowance: ¥ 50,000 a month
3) Housing allowance: up to ¥100,000 a month
   Apartments will normally be provided to awardees. The apartment floor area is 40 m^2 for awardees unaccompanied by their family and 60 m^2 for awardees to stay with their family in other than metropolitan areas such as Tokyo. If an awardee prefers to use a larger apartment because of the family size or otherwise ¥100,000 maximum per month may be paid as housing allowance; any shortfall is to be borne by the awardee.
4) International relocation allowance: ¥200,000
5) Travel allowance: ¥100,000 a year
   (within Japan)
6) Japanese language lesson courses are to be provided free of charge to the STA Fellowship awardees and their family members in Tsukuba area. Those who live in places other than the Tsukuba area will be entitled to reimbursement of Japanese language school tuition up to a specified amount.
7) Excursions or the like will be held to help make the Fellowship awardees and their family members in Tokyo/Tsukuba areas familiarized with Japan's culture, tradition and history.

In addition, ¥1,480,000 per year will be paid to the host institute to cover research costs and insurance for researchers will be paid by JISTEC during their stay in Japan to cover medical care.

APPLICATION PROCEDURE FOR STA FELLOWSHIP

Management of the STA Fellowship, including recruitment of candidates, is entrusted to the Japan International Science and Technology Exchange Center (JISTEC).

Responsible organizations overseas which represent the governments of their respective countries are given in Table 1.

A researcher wishing to be awarded the STA Fellowship should apply to the responsible organization in his/her country. Candidates are required to contact the desired host institute and obtain a letter of acceptance before filling an application with their government. Further information regarding the STA Fellowship and host Institutes is available from the responsible organizations.

A researcher whose country is not listed in Table 1 could contact a Japanese host institute directly, which in turn may recommend the reseacher to JISTEC as a candidate for STA Fellowship.

Fig. 1 shows the process from consult/contact to receipt of award.

(W6352)E

Fig. 1 PROCESS FLOW FROM CONSULT/CONTACT TO RECEIPT OF AWARD

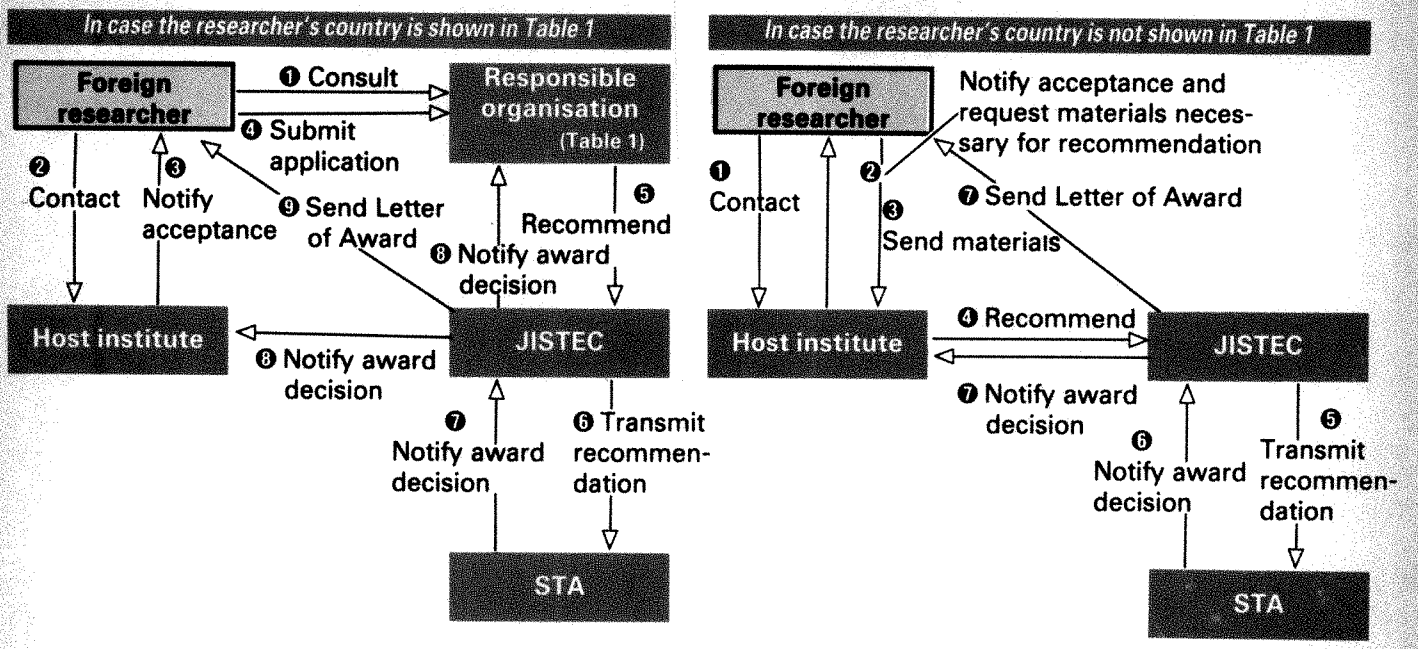

Table 1 RESPONSIBLE ORGANIZATIONS

Country	Contact
Australia	DEPARTMENT OF INDUSTRY, TECHNOLOGY AND COMMERCE The Secretary (Attention: Assistant Secretary, Japan Branch) GPO Box 9839, Canberra ACT 2601 Tel: 062-76-1000 Fax: 062-76-1122
Canada	NATURAL SCIENCE AND ENGINEERING RESEARCH COUNCIL Dr. R.J. Kavanagh Director-General (Scholarships & International Programs) Centennial Towers 200 Kent St., Ottawa, Ontario K1A 1H5
Federal Republic of Germany	ALEXANDER VON HUMBOLDT-STIFTUNG Dr. Rolf Hoffmann Selection Department Jean-Paul-Strasse 12 5300 Bonn 2, FRG Tel: (0228) 833-0 Fax: (0228 833-199
Finland	MINISTRY OF TRADE AND INDUSTRY Division for International Affairs Mr. Pertti Valtonen Head of Division Aleksanterinkatu 10, SF-00170 Helsinki
France	CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE Mr. Stuyck Taillandier Direction des Relations et de la Cooperation Internationales 15 Quai Anatole France, 75700 Paris Tel: 1-47 53 1515
Italy	MINISTERO DELLA RICERCA SCIENTIFICA E TECNOLOGICA Ufficio Relazioni Internazionali Dr. Mario Bove Director Lungotevere Thaon di Revel 76, 00100 Rome Tel: 6-369-941 Fax: 6-392-209 Tlx: 612548 RISCIE 1
Netherlands	MINISTRY OF EDUCATION AND SCIENCE Dr. ir. B. Okkerse Director-General for Higher Education and Scientific Research Dr. P. van't Klooster Deputy Director Division Research Organisations Directorate-General for Higher Education and Scientific Research P.O. Box 25000, 2700 LZ Zoetemeer
New Zealand	INTERNATIONAL SCIENCE UNIT Dept. of Scientific & Industrial Research Mr. M.A. Collins Assistant Director-General P.O. Box 1578, Wellington
Sweden	STYRELSEN FOR TEKNISK UTVECKLING Dr. Erik von Bahr Box 43200, 100 72 Stockholm Tel: 08-775 40 00 Fax: 19 68 26 Tlx: 10840 swedstu s
Switzerland	SWISS NATIONAL SCIENCE FOUNDATION Mr. Benno Frey Wildhainweg 20, CH-3001 Bern Tel: 031-24-54-24 Fax: 23-30-09 Tlx: 912-423
United Kingdom	THE ROYAL SOCIETY Ms. Karen Kimpton or Dr. Stephen Cox 6 Carlton House Terrace, London SW1Y 5AG Tel: 01-839-5561 Tlx: 917876
United States	NATIONAL SCIENCE FOUNDATION Dr. Charles W. Wallace Senior Program Manager US-Japan, Australia and New Zealand Programs Division of International Programs Washington, D.C. 20550 Tel: 202-357-9558
European Communities	THE COMMISSION OF THE EUROPEAN COMMUNITIES Mr. Giorgio Boggio, DG XII Head of Division, Rue de la Loi 200, 1049 Brussels Tel: 235-5635

QUESTIONS ABOUT STA FELLOWSHIP

Please direct questions about the fellowship scheme to the responsible organization in your country given in Table 1. If your country is not listed, inquiries will be received by JISTEC.

Japan International Science and Technology Exchange Center (JISTEC)

Address: Port One Building 6F, 1-7-6, Minato-machi
Tsuchiura City, Ibaraki Pref. 300
Japan
Telephone: 0298-24-3355
Facsimile: 0298-24-3214

THE LADY DAVIS FELLOWSHIP TRUST
P.O. BOX 1255, JERUSALEM 91904, ISRAEL
GRADUATE, POST-DOCTORAL AND PROFESSORIAL FELLOWSHIPS
AT THE HEBREW UNIVERSITY OF JERUSALEM OR THE TECHNION-ISRAEL INSTITUTE OF TECHNOLOGY, HAIFA
FOR 1990-91

APPLICATION INFORMATION: Lady Davis Graduate and Post-Doctoral Fellows are selected on the basis of demonstrated excellence in their studies and promise of distinction in their chosen fields of specialization. Graduate applicants may apply during their senior undergraduate year or after they have undertaken study in a graduate school. Post-doctoral applicants to the Hebrew University may apply not later than 3 years after completion of their doctoral dissertation. The Fellowships are tenable for one academic year and may be extended for another year. The grant covers travel, tuition fees and reasonable living expenses.

VISITING PROFESSORSHIPS are intended for candidates with the rank of Full or Associate Professor at their own institution. They are tenable from one semester to a full academic year. The grant includes a professorial salary and travel.

DEADLINE: November 30, 1989.

Requests from applicants (including Israelis) indicating category of Fellowship should be sent to above address.

(W6437)E

UNIVERSITY OF BRISTOL
DEPARTMENT OF GEOLOGY
POSTDOCTORAL FELLOWSHIP IN SEDIMENTOLOGY, SEDIMENTARY GEOCHEMISTRY OR HYDROGEOLOGY

The Geology department seeks to fill a three year post-doctoral fellowship in any branch of sedimentology, sedimentary geochemistry or hydrogeology. A modest amount of teaching is expected and the Department will assist with research expenses.

Salary will be on national Research and Analogous scales according to age, etc.

For further details telephone Bristol 303136 (ansaphone after 5.00pm) or write to the **Personnel Office, Senate House, University of Bristol, Bristol BS8 1TH.** Please quote reference A658. Please forward applications as soon as possible.

An Equal Opportunities Employer.

(1435)E

THE HEISER PROGRAM FOR RESEARCH IN LEPROSY

Beginning POSTDOCTORAL RESEARCH FELLOWSHIPS, RESEARCH GRANTS and VISITING RESEARCH AWARDS available in amounts up to $25,000 per year, plus other allowances. Applicants should have M.D., Ph.D., or equivalent degree. Applications by 1 February 1990, for awards to be activated June to December, 1990. For information write:

The Heiser Program,
450 East 63rd Street,
New York, NY 10021. (NW4117)E

POSTDOCTORAL FELLOWSHIP

(Farm Management, Lincoln University College)

Applications are invited for a two year Postdoctoral Fellowship in a research programme developing simulation models to investigate alternative farm production systems in New Zealand.

Applicants should be no more than 35 years of age with a Ph.D. in an Agricultural Production Science, Agricultural Economics or a related area. Excellent quantitative and analytical skills are essential. Experience with bio-economic simulation models and a familiarity with production agriculture will be advantageous.

The Fellowship is to be taken up before July 1990. Salary will be NZ$35,000 per annum. Airfares to New Zealand and assistance with removal expenses will be provided.

Applications close 15 September 1989. For further information contact the undersigned. When making enquiries please quote vacancy No. 89/31.

Registrar, Lincoln College, Canterbury, NEW ZEALAND. FAX (64) (3) 252–965.

(W6442)E

Intracellular Calcium Imaging/ Electrophysiology in Mammalian Neurones

MEDICAL RESEARCH COUNCIL

POSTDOCTORAL FELLOWSHIP

A three-year position is available immediately for a PhD to carry out in vitro work on the involvement of calcium binding proteins in regulation of cultured mammalian neurones involved in degenerative conditions such as Alzheimers and Huntingdons disease. Applicants should have interests in cell biology and physiology, and possibly previous experience in either patch clamp technology or ionic measurements in single cells. We are looking for a highly motivated, dynamic young scientist who is both imaginative and independent. The successful applicant will work in a well-funded research environment which has facilities for cell culture, real time video imaging and associated computing, photometric measurement of calcium and several electrophysiological rigs. The laboratory is close to Cambridge, and has numerous PhD students, post-docs and international visitors interested in the neurosciences. Applicants should provide: CV, list of publications and names and addresses of two referees and send by post to the Personnel Officer, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, or by fax (0223-833676). Salary will be commensurate with age and experience. For further information ring Dr. Mason or Dr. Emson on 0223-832312. (1447)E

STUDENTSHIPS

THE UNIVERSITY OF EDINBURGH
TREES AND ATMOSPHERIC POLLUTION

A three-year NERC Post-doctoral Research Fellowship is immediately available to investigate the uptake of pollutant materials through leaf surfaces. The work will involve low temperature SEM to quantify storm-damage to leaves, tracer techniques to investigate uptake from simulated cloudwater, and infra-red gas analysis to test the physiological performance of damaged leaves. The principal investigators are Drs. J. Grace and C. Jeffree. Candidates should have a Ph.D. in a relevant area, preferably with experience in SEM.

Applications should be sent to **Dr. J. Grace, Department of Forestry and Natural Resources, Darwin Building, King's Buildings, Edinburgh, EH9 3JU,** from whom further particulars can be obtained. The closing date for receipt of applications will be 21st September 1989.

PLEASE QUOTE REFERENCE NO. 5701 (1440)E

ROWETT RESEARCH INSTITUTE
Bucksburn, Aberdeen AB2 9SB

PhD STUDENTSHIP

A PhD Studentship is available for work on the molecular genetic analysis of polysaccharide degrading activities in the cellulolytic rumen bacterium *Ruminococcus flavefaciens*. The project will involve characterising the products of polysaccharidase genes that have been cloned in *E. coli* plasmid vectors, and studying the regulation of polysaccharidase gene expression in *R. flavefaciens* in response to changes in substrate availability.

Applicants should have an appropriate first or upper second class honours degree. The terms of the studentship will follow standard conditions for research council studentships, but will be supplemented to give an annual maintenance grant of £4,500. University fees will be met by the Institute. The studentship is available from 1 October 1989.

Applications including a CV and the names and addresses of two academic referees should be addressed to The Secretary, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, from whom further particulars can be obtained. Informal enquiries can be made to Dr H. Flint (0224 712751 ext 195).

Closing date: 22 September 1989. (1471)F

UNIVERSITY OF BRISTOL
DEPARTMENT OF OPTHALMOLOGY

RESEARCH STUDENTSHIP

Applications are invited for a PhD studentship in low temperature biology to investigate the effects of freezing and vitrification on the structure and function of mammalian corneas. The overall aim of the project is to develop a reliable method for the cryopreservation of human corneas for transplantation. For further information, please telephone 0272 507777, ext 60.

Candidates should have a first or upper second class honours degree in physiology, biochemistry or other biological subject.

Applications, quoting reference A672 and including curriculum vitae and two academic referees, should be made to **Professor D L Easty, Bristol Eye Hospital, Lower Maudlin St, Bristol, BS1 2LX**. Closing date two weeks after the appearance of this advertisement.

WE ARE AN EQUAL OPPORTUNITY EMPLOYER (1434)F

UNIVERSITY OF NOTTINGHAM
Department of Pharmaceutical Sciences

Research Studentship in Medicinal Chemistry

Applications are invited from suitably qualified graduates for a postgraduate research studentship leading to a PhD degree to investigate the chemical synthesis and computer-aided design of novel topoisomerase inhibitors as potential anticancer agents.

This studentship is being funded jointly by ICI Pharmaceuticals Division plc, and the Campaign for Cancer Research and is to start October 1st or as soon as possible thereafter.

Applications which include a CV and the names of two referees should be sent as soon as possible to **Dr D E Jackson, Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD** (0602 484848 ext. 3571) (1432)F

THE UNIVERSITY OF CALGARY

STUDENTSHIPS available in a newly formed Metastasis Research Group in the Faculty of Medicine. Areas of research include growth factor regulation of gene expression, molecular mechanisms of extracellular matrix-driven cell motility and proto-oncogenes and tested development. Deadline: October 31, 1989 for January 1990 enrolment.

Submit curriculum vitae, transcripts and two letters of reference to: **Ms. S. Berridge — Metastasis Research Group, Department of Pharmacology & Therapeutics, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta, Canada T2N 4N1.** (NW4121)F

AWARDS

Graduate Student Research Awards Ecology and Environmental Policy

The Science Division of the National Audubon Society invites applications for graduate student research support in ecology and environmental policy. Candidates should be at an early stage in their doctoral program.

Applications in two general areas are especially encouraged:

1. Field work on ecological processes with broad relevance to the management of National Audubon Society sanctuaries and other managed lands, for example on hydrological cycles, nutrient limitation, prescribed burning, or grazing impacts on natural communities;
2. Policy analyses of emerging national environmental issues, for example on the role of electric vehicles in moderating global climate changes, implications of biotechnology for land-use, using the permit-review process as a conservation tool, or comparing the conservation implications of food irradiation vs. pesticides.

These grants are part of an ongoing effort to expand the Society's scientific scope, therefore, proposals for ornithological research are ineligible for funding under this program.

Approximately three grants of up to $6000 apiece will be awarded. These grants will be renewable for a period of two to three years. In addition, a number of smaller, one-time grants may be awarded. Field research on Audubon sanctuaries may also be eligible for some on-site logistical support. Application guidelines may be obtained by writing:

Graduate Student Research Awards
National Audubon Society
950 Third Ave
NY, NY 10022

Completed applications are due by 15 February 1990. Winners will be announced by early May. (NW4112)N

ASSISTANTSHIPS

UNIVERSITY OF GLASGOW
Department of Biochemistry

Research Assistantships

Applications are invited for two Research Assistant positions in the Department of Biochemistry. The first position is to study the Biochemistry of Action of Interleukin 4. This post, funded by the Wellcome Trust, is for a period of 12 months in the first instance.

The second post, supported by the Medical Research Council, is to work on the role of immunoglobulin oligosaccharide groups in regulation of antibody effector functions. This position is for a period of 24 months.

Both posts are available immediately, and can be filled at either graduate (1B scale) or post-doctoral (1A scale) levels, and starting salary will be commensurate with age and experience. Please send Curriculum Vitae and names of two referees, by September 11th, 1989 to **Dr. W. Cushley, Department of Biochemistry, University of Glasgow, GLASGOW G12 8QQ (Tel: 041-339-8855, Extn. 5261).** (1457)P

COURSES

MSc IN MEDICAL IMMUNOLOGY: AUTUMN 1990 ENTRY
(University of London)

An intercollegiate 6-centre course

Applications are now invited for admission to the Sept/October 1990 entry onto the University's intercollegiate MSc Course in Medical Immunology. This is a part-time, two-year Course taught at the London University Medical Schools associated with St Thomas' Hospital, St Mary's Hospital, The Middlesex Hospital, King's College Hospital, The London Hospital and the National Heart and Lung Institute, Brompton Hospital. Additional teaching staff will come from other Schools and Institutes affiliated with the University. Teaching is given on one day per week and comprises lectures, clinicopathological and laboratory demonstrations, practical work and seminars, organised into 11 consecutive topics: (1) Introduction to Medical Immunology; (2) Molecular Immunology; (3) Cellular Immunology; (4) Autoimmunity and disease; (5) Arthritis, nephritis and vasculitis; (6) Extrinsic allergic disease; (7) Infective diseases; (8) Immunodeficiency; (9) Transplantation; (10) Immunology of neoplasia; (11) Principles of diagnostic medical immunology, quality control and laboratory management. During the two years students also undertake a research project.

The Course is open to U.K. and overseas graduates in Medicine, Veterinary Medicine, Dentistry, Pharmacy or Biological Sciences, who are associated with a London University Teaching Institution. Overseas applicants can be advised on appropriate attachments in London. Examinations will be held at the end of the second year.

Successful completion of the Course will qualify the candidate to apply to the Royal College of Pathologists for training credit towards the total requirements for the MRCPath. The Course is also approved for Study Leave under Regulation HM (67) 27 for UK medical graduates working in the Health Service.

Further particulars, including the course syllabus and application form, are obtainable from the **Secretary, Department of Immunology, UMDS, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, United Kingdom.** (1452)C

continued on page 22

SERVICES

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FONDAZIONE SIGMA-TAU

KRAEPELIN'S WORKSHOP

16-17 September 1989
Grand Hotel Des Iles Borromées
Stresa (Novara) - Italy

The renewed interest in the classification of illness in the sphere of neuropsychiatry has stimulated a rediscovery of the work of Emil Kraepelin. By the definition of dementia praecox and manic depressive psycosis, Kraepelin's work has guided the development of neuropsychiatry through its ramifications. Beginning this year and in September of each year thereafter, Foundation Sigma-Tau will sponsor a meeting in honor of Emil Kraepelin in Stresa (Novara - Italy) where he owned a house to which he retreated for his studies.
The theme of the 1st Emil Kraepelin Workshop on September 16-17 1989 is "DEMENTIA".

Saturday 16th September 1989
10 am - 12.30 pm

THE INFLUENCE OF EMIL KRAEPELIN ON CLINICAL NEUROPSYCHIATRY

Chairman:
L. Ravizza (Torino)
Discussant:
Sir M. Roth (Cambridge), V. Conde Lopez (Villadolid), C.L. Cazzullo (Milano), H. Hippius (Munich), T.A. Ban (Nashville).

3 - 6 pm

"WHAT DEMENTIA MEANS TO THE SPECIALIST?"

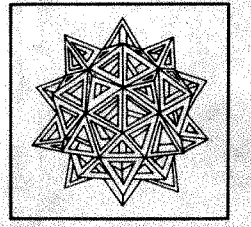
Chairman:
G. Baumgartner (Zurich)
Discussant?
C.G. Gottfries (Gothenburg):
"Dementia: the neurochemists viewpoint"
V. Chan Palay (Zurich):
"Dementia: a disease of neuronal communication breakdown in neurotransmitter systems"
A. Oliverio (Roma):
"A neurophysiological and pharmacological approach to Dementia"
H. Hippius (Munich):
"Dementia: from the psychiatrists standpoint"
J.M. Martinez-Lage (Pamplona):
"Early Diagnosis in Dementia"
K.L. Leender (Villigen):
"PET studies in Dementia: present findings and future perspectives"

Sunday, 17th September 1989
10 am - 12.30 pm

Chairman:
A. Agnoli (Roma)
Presentation of "Memories" by Emil Kraepelin
G. Kantza and P. Kantzas (eds.)

General discussion

Contact: **Dr. Bartolomucci**
Sigma Tau S.p.A.
Via S. Caterina da Siena
46-00186
Roma, Italy
Tel: 0039 6 6783458
6841529
6841601
Fax No: 0039 6 6790745



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Subject

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E1	Biology
F1	Chemistry
G1	Earth Sci./Geo
H1	Environ. Sci.
I1	Engineer/Math
J1	Med. Research
K1	Microbiology
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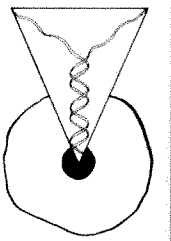
GENE MANIPULATION IN BIOLOGY AND HUMAN DISEASE

9–10 November 1989 at the Copley Plaza Hotel, Boston, MA

BOOK BEFORE 31 AUGUST TO SAVE $75 WHILE PLACES REMAIN



How readily can the genes responsible for predisposition to conditions such as ischaemic heart disease or schizophrenia be identified? What are the prospects of human gene therapy? Are transgenic animals the key to understanding the precise genetic control of development? How much more sophisticated can methods of genetic manipulation and gene identification become?

These are among the major questions that will be addressed by the distinguished speakers at Nature's 1989 conference. A chairman will outline the context and aims of each session, the speakers will review their area of expertise and report the latest advances, and a rapporteur will draw out the implications of each session. There will be a panel discussion of the ethical and legal problems raised by human gene research and therapy. For the convenience of delegates, the second day of the conference will finish in mid-afternoon, whereas the first day will be extended by the panel session followed by a wine and cheese reception.

We expect the conference to sell out well before opening, so urge you to make sure of your place now by returning the form below. TO SAVE $75 BOOK BEFORE 31 AUGUST

CONFERENCE SESSIONS

1 HUMAN GENETIC DISEASE

James Crow, U. of Wisconsin (chairman)
Louis Kunkel, Harvard Medical School
Gerd Utermann, University of Innsbruck
Kenneth Kidd, Yale University
Eric Lander, Whitehead Institute (rapporteur)

2 PROSPECTS FOR GENE THERAPY

Leon Rosenberg, Yale (chairman)
Irving Weissman, Stanford University
Richard Mulligan, Whitehead Institute
French Anderson, NIH
David Baltimore, Whitehead (rapporteur)

3 HUMAN GENE RESEARCH: PANEL DISCUSSION

4 TRANSGENIC MOUSE MODELS

Richard Palmiter, Washington U. (chairman)
Douglas Hanahan, University of California SF
Erwin Wagner, IMP Vienna
Ron Evans, Salk Institute
Brigid Hogan, Vanderbilt U. (rapporteur)

5 GENE DISRUPTION AND DEVELOPMENT

Mario Capecchi, U. of Utah (chairman)
Fred Alt, Columbia University
Janet Rossant, University of Toronto
Peter Gruss, Max Planck Institute Gottingen
Bruce Alberts, U. of California SF (rapporteur)

GENE MANIPULATION IN BIOLOGY AND HUMAN DISEASE: 9–10 November 1989

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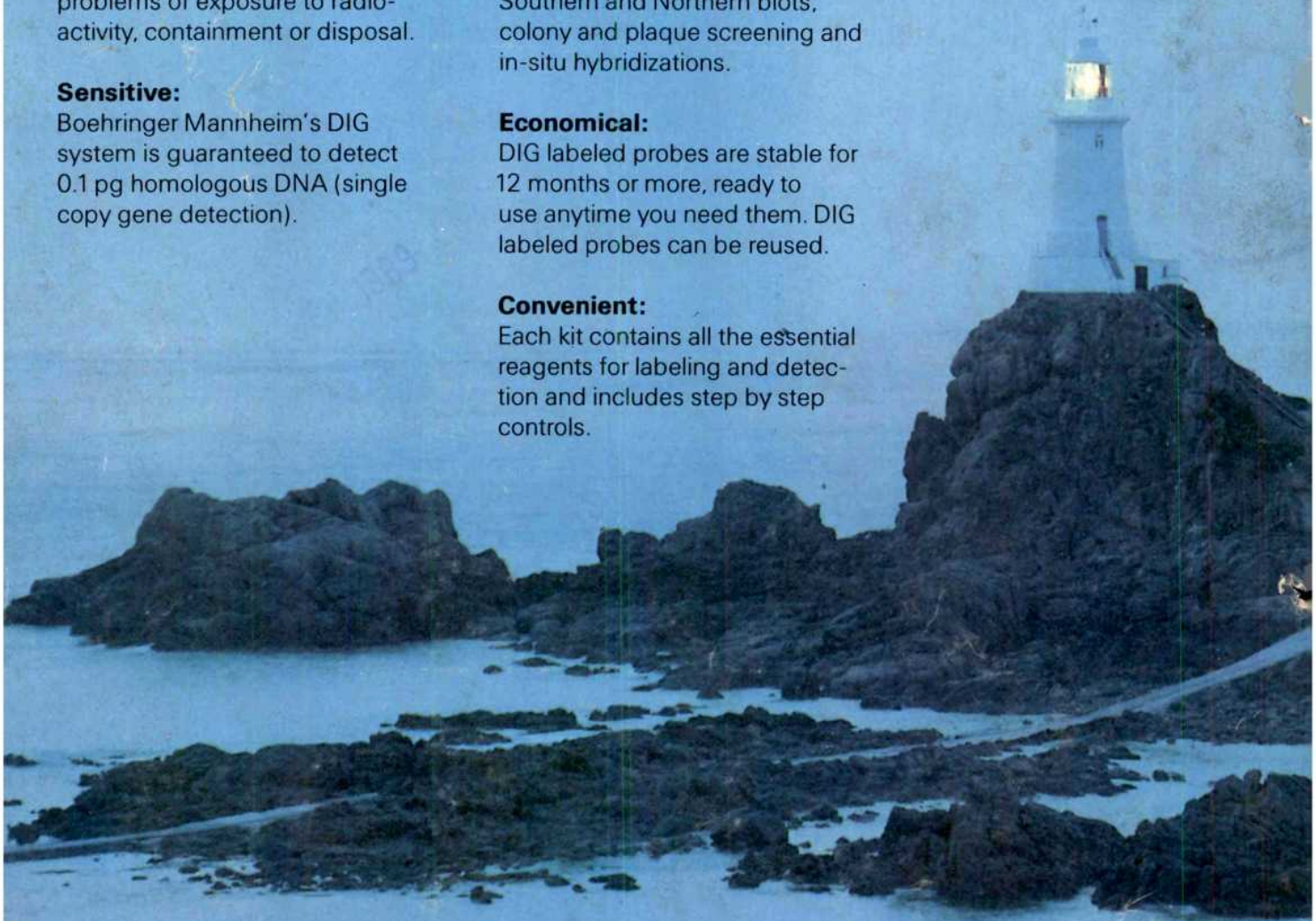

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